Chemical Signals in Arthropods – New structural Motifs

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina

zu Braunschweig

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

genehmigte

D i s s e r t a t i o n

von Stephan Goller

aus Hildesheim

1. Referent: Professor Dr. Stefan Schulz
2. Referentin: Professor Dr. Monika Mazik
eingereicht am: 16.01.2008
mündliche Prüfung (Disputation) am: 04.04.2008

Druckjahr 2008

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Fakultät für Lebenswissenschaften, vertreten durch den Mentor dieser Arbeit, in folgenden Beiträgen vorab veröffentlicht:

Posterbeiträge:

09/2004	ORCHEM in Bad Nauheim, Titel: Chiral siloxanes for the gaschromatographic analysis of natural diols
09/2005	1.Deutsch-Französischer Kongreß für Organische Chemie in Goslar, Titel: New chiral silylating reagents for the determination of the absolute configuration of natural diols
07/2006	22.Kongreß der „International Society of Chemical Ecology“ in Barcelona, Titel: Biosynthesis of (3*Z*,6*Z*,9*Z*)-octadecatriene: A compound of the pheromone blend of *Erannis bajaria*
07/2007	23.Kongreß der „International Society of Chemical Ecology“ in Jena Titel: Altered pheromone blend of the winter moth *Erannis bajaria* (Lepidoptera: Geometridae)

Vorträge:

07/2005	Organisch-Chemische Kolloquium des Instituts für Organische Chemie der Technischen Universität Braunschweig, Titel: Chirale Silylierungsreagentien für die gaschromatographische Analyse natürlicher Diole
12/2006	GDCh-Kolloquium Ortsverband Braunschweig, Titel: Strukturaufklärung neuartiger Spinnenlipide aus *Argyrodes elevatus*

Veröffentlichung:

S. Goller, G. Szöcs, W. Francke, S. Schulz 2007.
Biosynthesis of (3*Z*,6*Z*,9*Z*)-octadecatriene: A compound of the pheromone blend of *Erannis bajaria* (Lepidoptera: Geometridae). *J. Chem. Ecol.* 33:1505-1509.

Danksagungen

Als erstens möchte ich mich bei meinem Mentor, Herrn Prof. Dr. Stefan Schulz, für die Aufnahme in den Arbeitskreis, für die Bereitstellung des interessanten Themas und die Begutachtung dieser Arbeit bedanken. Durch seinen Führungsstil hat er es mir ermöglicht, diese Arbeit in einem großen wissenschaftlichen Freiraum unter ständiger Gesprächsbereitschaft zu entwickeln. Dadurch konnte ich meine Persönlichkeit deutlich erweitern, was mich auch nun in meiner weiteren beruflichen Entwicklung voranbringt. Auch möchte ich erwähnen, daß er die Korrekturen zu dieser Arbeit sehr schnell erledigt hat, was ich bei all seinen Verpflichtungen nicht für selbstverständlich halte.

Daneben bedanke ich mich bei Frau Prof. Dr. Mazik, Frau Prof. Dr. Mischnick und Herrn Prof. Dr. Gericke für die Begutachtung meiner Arbeit und die Teilnahme an der Prüfungskommission.

Des Weiteren gilt mein Dank Herrn PD Dr. Jahn für die gute Zusammenarbeit während der Praktika Organische Chemie für Biotechnologen. Durch die Überlassung vieler Kompetenzen konnte ich auch hier mein Profil erweitern.

Außerdem gilt mein Dank den Kooperationspartnern, Prof Dr. Franke, Prof. Dr. Gnatzy, Prof. Dr. Schütz, Prof. Dr. Riechert, Dr. Francke, Dr. Szöcs, PD Dr. Uhl, Dr. Weissbecker, S. Funke und A. B. Payne, die durch ihre Mitarbeit die Arbeit in diesem Umfang möglich gemacht haben.

Für eine wohltuende Arbeitsatmosphäre möchte ich mich auch bei meinen ehemaligen Kollegen aus der Arbeitsgruppe bedanken, namentlich:
Elena Barcari, Dr. Katalin Böröczky, Dr. Gregor Brasse, Birte Flachsbarth, Matthias Fritzsche, Christopher Häberlein, Andreas Hennig, Nico Klewer, Dr. Karsten Krückert, Jasmin Müller, Dr. Edyta Nagrodzka, Nicole Schlüter, Andreas Schmidt, Thies Schulze, Paul Sobik, Verena Thiel, Nicole Tiesing und Frau Weiß.
Besonders hervorheben möchte ich folgende Personen:
Wiebke Jacobs und Selma Yildizhan danke ich für eine gute Zusammenarbeit bei den Arbeiten zu der chiralen Analyse natürlicher Diole.
Satya Chinta danke ich für eine gute Zusammenarbeit bei den Arbeiten zur Lipidanalytik bei *Argyrodes elevatus*.

Björn Hahn und Thorben Nawrath möchte ich danken, da sie mich während meiner Dissertation in den Zeiten, als ich mit Lehraufgaben beschäftigt war, in der Betreuung meiner Praktikanten unterstützt haben. Nur so konnte ich in dieser Zeit die praktischen Arbeiten an meiner Dissertation konsequent weiterführen.
Dr. Jeroen Sidney Dickschat danke ich für umfangreiche wissenschaftliche Diskussionen.

Entscheidend für die Entwicklung dieser Arbeit waren auch zahlreiche Praktikanten. Bei diesen möchte ich mich auch bedanken.

Frau Gödecke, als Vorsitzende des Freundeskreises des Instituts für Organische Chemie (FIOC), danke ich für die finanzielle Unterstützung. Nur dadurch konnte ich an wissenschaftlichen Tagungen teilnehmen und Teile meiner Ergebnisse in einem wissenschaftlichen Forum präsentieren.

Ebenso gilt mein Dank dem Glasbläser, sämtlichen Mitarbeitern der MS-Abteilung, der NMR-Abteilung und des zentralen Chemikalienlagers.

Meinem Onkel, Karl-Heinz Hodur, danke ich für das Binden dieser Arbeit.

Abschließend gilt mein größter Dank meinen Eltern, meinem Bruder und meiner Oma, die mich durch ihre Persönlichkeit auf diesem Weg begleitet haben.

Danke !

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1. Introduction

Semiochemicals play an important regulative role in the life of insects in addition to other communication channels as optic, acoustic, or tactile communication. Semiochemicals include allomones, kairomones, and pheromones.

Allomones support the interspecifc communication. These compounds are advantageous for the emitter and are often involved in the chemical defense of insects. Poison glands of hymenoptera or the poisonous hairs of butterfly caterpillars exhibit important reservoirs for these allomones. In contrast, kairomones are disadvantageous for the emitter; insects with a parasitic lifestyle use these semiochemicals released by the host for their location.

Pheromones control the intraspecific communication between the sexes of insects and are essential for the regulation of reproduction. Mainly, the females release pheromones for male attraction. But there are also known male pheromones. Pheromones can be subdivided by their range. Long-range pheromones are necessary for the attraction of conspecifics over a longer distance.

On the other hand, hydrocarbons as main constituents of the lipid profile of insect cuticles can be important as close-range pheromones. Their chain length ranges from 21 to 45 carbon atoms and the occurrence of methyl branches increases additionally their variability [Nelson and Blomquist, 1995]. In solitary and especially in social insects, hydrocarbons serve as contact pheromones for kin recognition [Howard, 1993]. In the termite species *Macrotermes subhyalinus*, differentiation between non-nestmates and nestmates is evoked by the cuticle hydrocarbon profile [Kaib *et al.*, 2004]. Perception of a non-nestmate hydrocarbon profile causes aggressive behaviour against these heterospecific individuals. A caste-specific hydrocarbon profile is observed in the termite *Captotermes formosanus* [Haverty *et al.*, 1996]. The hydrocarbon profile of each caste consists predominantly of *n*-alkanes and monomethyl branched alkanes. The composition of extracts of the different castes resembles each other qualitatively. Otherwise, a quantitative analysis of the hydrocarbon profiles showed that the caste-affiliation bases on quantitative differences in the hydrocarbon profiles. In solitary insects, a sexual dimorphism of the composition of the hydrocarbon profile occurs often [Singer, 1998]. Male courtship behaviour in the solitary wasp *Cardiochiles nigriceps* is induced after perception of the female hydrocarbon profile. Frozen males, covered with an extract of the female cuticle, were as attractive as females in manipulation experiments [Syvertsen *et al.*, 1995]. Hydrocarbon profiles in the house fly *Musca domestica* also differ between the sexes and the female hydrocarbon blend contains (*Z*)-9-tricosene as sex pheromone [Carlson *et al.*, 1971]. In contrast, a male-derived polymethylalkene is present on the cuticle of tsetse flies (*Glossina* spp.) which is transferred to the female cuticle as anti-aphrodisiac during copulation to prevent ineffective further mating attempts

[Carlson and Schlein, 1991]. Furthermore, the importance of the hydrocarbon profile as communication channel is emphasized by the observation that inquilines mimic the hydrocarbon profile of their hosts to suppress aggressive behaviour [Dettner and Liepert, 1994].

Saturated and unsaturated hydrocarbons are also important butterfly sex pheromones. Often the biosynthesis of these hydrocarbons is elusive in insects and therefore, butterflies represent nice model organisms for biosynthetic investigations because knowledge about the mechanisms of their formation are advanced in these insects [Jurenka, 2004]

Alkanediols exhibit another widespread class of natural compounds in arthropods present as constituents of the cuticular lipid blend. These compounds can also occur in plants [Schulz *et al.*, 2000]. The role of these compounds for arthropds and plants is still unknown. An implication in the semiochemistry of insects similar to hydrocarbons seems to be possible. Recently, isomeric heptacosanediols with an 1,2-, 1,3- and 1,4-arrangement of the hydroxy groups were identified in wing extracts of the big and small cabbage white butterflies *Pieris brassicae* and *Pieris rapae* [Yildizhan, personal communication]. Alkanediols with hydroxy groups in position 1 and 3 are common compounds in cuticular extracts of spiders. A blend of them with a chain length between 20 and 22 carbon atoms occurred in a cephalothorax extract of the american desert spider *Agelenopsis aperta*. 1,3-Docosanediol was the dominant member of this mixture. Besides, these diols are also part of cuticular extracts of *Heliconius* butterflies [Yildizhan, personal communication]. All these diols are chiral and up to four stereoisomers are possible if both hydroxy groups are internally arranged. The absolute configuration (AC) of these diols can be important for their bioactivity and thus, reliable methods allowing the determination of their ACs are needed. Normally, these diols are part of a complex mixture of natural compounds and often only a low amount of material is available for the chiral analysis. Therefore, new chiral analysing methods are necessary to investigate their stereochemistry. After assignment of their ACs, reference compounds with the correct stereochemistry can be applied to investigate their influence for the semiochemistry of insects.

These examples demonstrate that the chemical analysis of the cuticular lipid profile is important to get insight into the chemical communication of insects. The influence of the hydrocarbons is already quite well understood, but like the diols, there are other compound classes with unknown functionality and thus, further investigations are valuable.

Besides the cuticle lipid profile, the secretion of glands represents another important source for semiochemicals in insects. Especially, the hymenoptera have diverse glands and they are dispersed over the whole insect body [VanderMeer, Breed, Winston, and Espelie, 1998]. Many glands are located in the head region including mandibular gland, maxillary gland, postpharyngeal gland,

infrabuccal sac, and pharynx and the function of their secretion is well-known [Attygalle and Morgan, 1984]. The Dufour gland (DG) is a common trait of female hymenoptera and exhibits another gland type of these insects. But the function of its secretion is non-uniform [Billen, 1987]. The aphidae and vespidae in the hymenopteren suborder apocrita have a joint between DG and vaginal tract suggesting a function in reproductive behaviour. In contrast, morphological investigations in the formicidae, another subfamily of the apocrita, proved close interaction between the DG and the sting base. In this case, the DG is thought to serve as reservoir for compounds involved in the chemical communication of these species. Hydrocarbons are also often constituents of these glands.

The chemical analysis of the secretion of these numerous insect glands is in addition to the investigation of the chemical composition of the cuticle lipid profile essential for the understanding of the chemical communication in insects.

The semiochemisty of spiders (araneae) is less investigated compared to the semiochemistry of insects. Up to now, only three spider pheromones were identified [Schulz and Toft, 1993; Papke *et al.*, 2001; Tichy *et al.*, 2001]. In contrast, investigations about pheromones in spiders are numerous, but the structures of these semiochemicals are mostly unknown [Schulz, 2004]. Often, knowledge about chemical cues in the communication of spiders bases on the positive response of one sex to cuticule or web extracts of the opposite sex in bioassays.

The lipid profile of spiders resembles often the chemical composition of cuticular blends of other arthropods with unbranched and branched alkanes as most important lipid class [Schulz, 1997b]. Minor lipid classes are represented by alcohols, aldehydes, and acids [Schulz, 2004].

On the other hand, spiders are able to biosynthesize lipids other than hydrocarbons. Spiders are carnivorous and they feed on insects. Therefore, it was proposed that the lipid profile of the spider cuticle differs from that of insects to prevent the alteration of their lipid blends by residues of the food item [Schulz, 2004].

1-Methoxyalkanes were reported as important part of the lipid blend of the spider *Nephila clavipes* [Schulz, 2001]. The chain length ranges from 25 to 45 carbon atoms and these derivatives contain up to three methyl branches. Besides *N. clavipes*, other spider species contain these derivatives on their cuticle with and without methyl branches. They were identified in several species of linyphiids. Derivatives with an odd- or even number of carbon atoms in the chain, with a methyl group at C-2, and an additional methyl branch in (ω-2)- or (ω-3)-position are predominant in *Linyphia triangularis* [Schulz and Toft, 1993]. Unbranched derivatives occur in the red widow spider *Latrodectus rivivensis* and these derivatives triggered searching behaviour in males as

γ-aminobutyric acid did [Papke, Schulz, and Lubin, unpublished results]. *n*-Propyl esters of multiple branched long-chain acids are present in the social spider *Anelosimus eximus* and the composition of the lipid blends differs between colonies, suggesting that they are an important fingerprint of the colony, similar to hydrocarbons in social insects [Bagnères *et al.*, 1997].

As reported in insects, the role of hydrocarbons in spiders may be similar. Fatty acids are involved in the aggression behaviour in the spider *Tegenaria atrica* [Pourié *et al.*, 2005]. Spiderlings of *T.* a*trica* live either solitary or gregarious and females of this species tend to tolerate gregarious spiderlings whereas aggression is elicited towards solitary spiderlings. Chemical analysis and a behavioural assay demonstrated that different cuticular lipid profiles are responsible for this behaviour. Experiments revealed that aggression is associated with an increased amount of hexadecanoic acid and linoleic acid in the lipid blend of solitary spiderlings. Another study dealt with the chemical composition of the web droplets from the orb-weaver spider *N. clavipes* [Salles *et al.*, 2006]. These droplets contain several saturated and unsaturated fatty acids in the range of 10 and 20 carbon atoms as well as toxic proteins. Toxicity of the acids for prey was demonstrated by topical application whereas proteins alone were ineffective. In contrast, a topical applied mixture of acids and proteins increased the toxicity compared to the acids alone.

These examples indicate that spiders also rely on chemical communication as insects, but the understanding of this communication channel is scarce. Therefore, work about the semiochemistry of spiders is needed.

The following chapters of this thesis investigate these topics and try to add to the semiochemistry of wasps and spiders.

2. Chemical analysis of the parasitic wasps *Ampulex compressa* and *Liris niger*

2.1 Aim

This chapter deals with the comparative chemical analysis of the cuticular lipid profile of males and females of the jewel wasp *Ampulex compressa* as well as with the lipid profile of the female Dufour gland (DG). The chemical composition of dichloromethane (DCM) extracts was elucidated by GC-MS analysis. If necessary, extracts were derivatized prior to GC-MS analysis to obtain more structural information about the constituents of these blends. Interesting compounds putatively involved in the semiochemistry of this species were synthesized to verify structure proposals and to make them available for biotesting in cooperation with Prof. Gnatzy (University of Frankfurt).

Furthermore, the volatiles emitted by males, unmated females, and mated females of the digger wasp *Liris niger* were investigated. A striking sexual dimorphism in the morphology of the antennae was observed and male antennae are characterized by numerous sensilla trichodea type 3 responsible for olfactory perception [Gnatzy *et al.*, 2006]. In contrast, this sensilla type occurs sparsely on female antennae. These results were the motif for headspace (HS) investigations because it was questioned whether females produce volatile semiochemicals which males recognize by their antennae, followed by triggering a male-specific behavioural trait. A detailed analysis of the male and female cuticular lipid profile as well as of the DG was published recently [Gnatzy *et al.*, 2004]. The volatiles were collected by open loop stripping analysis (OLSA; section 8.2), followed by GC-MS analysis. Like above, the chemical composition of these extracts was compared and interesting compounds were synthesized for biotests. Biotests were perfomed by Prof. Gnatzy as well as Prof. Schütz, and Dr Weissbecker (both from the University of Göttingen).

2.2 Taxonomy of wasps and their lifestyle

Wasps rank among the order of hymenoptera and important information about taxonomy and their lifestyle are summarized in text books of entomology [e. g. Dettner and Peters, 2003]. This order consists of wasps, ants, bees, and sawflies. This class of insecta is very rich in the number of species and at least 115,000 species are described [Westheide and Rieger, 1996]. Pollination is the most important task of species in this order [Keeling, Plettner and Slessor, 2004].

Furthermore, hymenopteren species are able to control different insect pest species. The wasp *Agrypon flaveolatum* parasitises other insects and uses them as hosts for their offspring. This species was intentionally introduced to control the winter moth *Operophtera brumata* in Nova Scotia (Canada) [Embree, 1966]. Originally exclusively located in Europe, *O. brumata* causes

damage in numerous deciduous trees. After attaching the eggs on the host, emerging larvae of *A. flaveolatum* feed on the host and hence, parasitising insects are a nice tool for the control of important insect pests.

In contrast, other hymenopteren species by themselves are disadvantageous for plants because they employ them as nutritional resource for their larvae like plant wasps (symphyta) focusing on conifers. The selection of the host plant is often not very elaborated and they prefer trees and shrubs. Other important plant pests focus on fruit trees like larvae of the species *Hoplocampa testudinea*. This species is detrimental for apple trees and it is one of the major pest species for apple orchards north of the Alps [Graf *et al.*, 2002].

In general, hymenoptera are subdivided into two suborders called symphyta and apocrita. The suborder of symphyta comprises the described phytophageous plant wasps and includes species of sawflies and horntails [Quicke, 1997]. In contrast, species of the suborder apocrita are entomophageous and include ants, bees and wasps. Furthermore, it is distinguished between aculeata and parasitica in this suborder. The group aculeata comprises sting wasps and species of parasitica subdue other arthropods for the grow up of their offspring. The two species of interest in this chapter, *A. compressa* and *L. niger*, are part of the suborder apocrita. The jewel wasp *A. compressa* depends on the cockroach *Periplaneta americana* as host whereas the digger wasp *L. niger* selects the cricket *Acheta domesticus*. Parasitism, an interaction only beneficial for the parasite, occurs in both suborders of the hymenoptera and it is defined as the subjugation of an organism (host) by another organism (guest).

In more detail, entomophageous insects can be subdivided into three groups depending on the behaviour against their host. Robbers kill immediately their host. Parasites, mainly the larvae of this species, consume parts of their host without dispatching them. Parasitoids like *A. compressa* and *L. niger* exhibit an intermediate between robbers and parasites because their behaviour is initially similar to parasites but finally the host dies if the parasite has accomplished his development. An *A. compressa* male is depicted in Figure 1 as well as further pictures showing the interaction between *A. compressa* larvae and the cockroach *P. americana*. During the subjugation, the host is immobilized by two stings of *A. compressa* females [Haspel *et al.*, 2003]. The first one is directed to the thorax and represses escape behaviour of the cockroach but the poisonous action is only temporary. The second sting aims at the subesophageal ganglion, a part of the cerebral nervous system (CNS) in arthropods, and long-time lethargy results. Furthermore, this sting induces intensive grooming behaviour in *P. americana* probably caused by the stimulation of the neural center responsible for this behavioural pattern. Then, the cockroach is transported to the brood place and eggs are attached on the hip of the midlegs of the host. Eclosed larvae suck on the

body fluid of the host and finally they consume the innards of the dying cockroach. Pupation follows and development to adult individuals is finished after 40 to 45 days. The hunting behaviour of *L. niger* (Figure 2) is very similar. The host suffers from paralysis and its metabolic rate is decreased [Roces and Gnatzy, 1997; Ferber *et al.*, 1999].

Other species in the order hymenoptera are characterized by a solitary or a social lifestyle. *A. compressa* and *L.* niger are both solitary. On the other hand, termites (isoptera), ants, some wasps of the family vespidae, and some bees are adapted to a social lifestyle. Traits of social lifestyle are fulfilled by a cooperation in brood care, sharing reproductive tasks (caste determination) and overlapping generations. The impact of acoustic, tactile, and olfactory signals regulates the different tasks in a colony. Olfactory communication is the most important mode and the involved structures of semiochemicals as well as their different functions are versatile. Semiochemicals trigger aggregation, support species recognition, or mediate mating behaviour between the sexes.

Figure 1: Left picture: *Ampulex compressa* male climbing on a twig. Picture in the middle: Attached egg on the hip of the midleg of *Periplaneta americana*. Right picture: Eclosed larvae feeding on the host. This species has its origin in India. Pictures by Aquazoo Düsseldorf, Insect Department.

Figure 2: Female of *Liris niger*. This species is located in the mediterranean zone. The picture was made by Prof. Gnatzy.

2.3 Semiochemicals and mating strategies of hymenoptera

Semiochemicals in the order hymenoptera are very versatile and therefore the following discussion focuses on recently identified sex pheromones of parasitic hymenoptera [Keeling, Plettner and Slessor, 2004]. Some examples are compiled in Figure 3.

Sex pheromones act either as close-range or long-range pheromones. Female calling is the predominant system of long-range attraction in the suborder parasitica but semiochemicals produced by both sexes are important in closed-range interactions [Quicke, 1997].

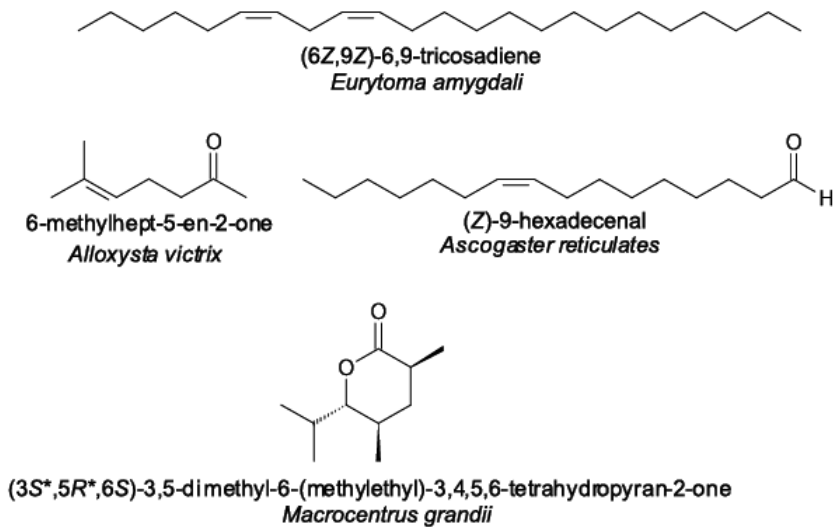

Figure 3: Compilation of recently identified sex pheromones in parasitic hymenoptera [Keeling, Plettner and Slessor, 2004].

The almond wasp *Eurytoma amygdali* is harmful for almonds because mated females deposit their eggs in the unripe fruit and larvae consume then the endosperm of the fruit. Females of this species attract males by a mixture of (6Z,9Z)-6,9-tricosadiene (6*Z*,9*Z*-23:H) and (6Z,9Z)-6,9-pentacosadiene (6*Z*,9*Z*-25:H) [Krokos *et al.*, 2001]. The aphid hyperparasitoid *Alloxysta victrix* reduces the negative impact of aphids parasitising cereal fields because hyperparasitoid larvae occur as endoparasites in the aphids. Investigations showed that sulcatone, 6-methyl-5-hepten-2-one, is able to attract males whereas this compound is repellent for females [Micha *et al.*, 1993]. Females of the parasitoid *Ascogaster reticulates* produce (*Z*)-9-hexadecenal in their tibial glands and males are guided to females by depositing the pheromone on substrates [Kainoh and Oishi, 1993]. This species is an egg-larval parasite of *Adoxophyes* spp. (tortricidae) that attacks apple trees. The lactone (3*S**,5*R**,6*S*)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one is part of the female pheromone blend of *Macrocentrus grandii* and this species parasitises the butterfly *Ostrinia nubilalis*, an important pest of maize plants

[Swedenborg *et al.*, 1992]. All these examples demonstrate that the investigation of the semiochemistry in parasitic hymenoptera is important because these species can be potentially used for the control of agricultural insect pests.

Besides sex pheromones, parasitic hymemoptera utilize often external or internal host marking pheromones. These compounds signalize an infested host and avoid oviposition by other conspecific females. Furthermore, these compounds are able to impair the development of the host. Juvenile hormone (JH) II and JH III, illustrated in Figure 4, fulfill this role because they avoid oviposition by other females of *Dendrocerus carpenteri* and cause developmental delay in the host *Aphidius uzbekistanicus* [Höller *et al.*, 1991; Höller *et al.*, 1994].

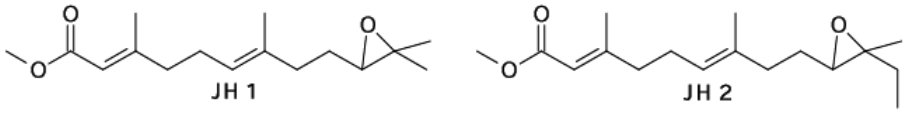

Figure 4: Structures of juvenile hormone 1 and juvenile hormone 2.

Furthermore, some parasites locate their host by perception of its semiochemicals. The egg-parasite *Trichogramma evanescens* detects the stored product pests species *Ephestia* spp. and *Plodia interpunctella* by recognition of their sex pheromones [Schöller and Prozell, 2002]. Another mode of host recognition bases on the perception of host frass volatiles as decribed in the parasite wasp *Cotesia plutellae*. This species parasitises the diamondback moth *Plutella xylostella* and perceives the host sex pheromones as well as the host frass volatiles consisiting of dipropyl disulfide, dimethyl disulfide, allyl isothiocyanate, and dimethyl trisulfide [Reddy *et al.*, 2002].

The mating behaviour in the order hymenoptera is very complex [Ayasse, Paxton and Tengö, 2001; Paxton, 2005]. In general, mating systems are classified by the location where the sexes meet. This location is named rendezvous site and three groups are differentiated: 1) female emergence site 2) resource based rendezvous site and 3) non-resource based rendezvous site.

Female emergence sites include overwintering sites, sleeping sites, and oviposition sites of females. Virign females of the nesting bee, *Colletes cunicularius*, are carved into the soil at their emergence site and release linalool. After perception, males react with increased flight activity and they move towards the emergence site. After arrival, they start to excavate the virgin females [Cane and Tengö, 1981]. Males of the bumblebee wolf *Philanthus bicinctus* also perceive a female-derived pheromone which triggers male-male competition at the rendezvous site [Gwynne, 1980]. Loser in this competition evade to potential forage sites of females and try to

defend these places. Mating success in this species is correlated with the male body size and larger males are preferred. The mating behaviour of *P. bicinctus* is defined as territorial behaviour at the rendezvous site. In contrast, male seeking behaviour of *C. cunicularius* is characterized as non-territorial behaviour at the rendezvous site.

Furthermore, mating takes place at non-territorial and territorial resource-based rendezvous sites and especially flowers as potential foraging sites are important. Non-territorial male behaviour is illustrated in males of the red mason bee *Osmia rufa*. Females of this species are difficult to locate because they are dispersed at foraging places in the environment and in this case, non-territorial mating behaviour is appropriate to increase mating success [Seidelmann, 1999]. On the other hand, females of the bee *Anthidium manicatum* forage at garden plants and territorial-defending male-male competitions are performed at these sites. Male-mating success in this species is correlated with the body size and larger males are preferred. Usurpation of other male territories also takes place [Severinghaus *et al.*, 1981].

Additional rendezvous sites are non-resource based and include landmarks or fly ways with nonterritorial and territorial male mating strategies. Non-territorial behaviour is observed in harvester ants of the genus *Pogonomyrmex* and mating sites are grounds, bushes, and trees [Hölldobler, 1976]. Here, male-derived mandibular gland products induce the aggregation of males as well as the appearance of virgin females. Then, courtship behaviour is induced by the release of a female pheromone. In contrast, males of the central american bee *Centris adani* reflect territorial non-resourced-based mating behaviour and they release nerol, geraniol, neral, ethyl laurate, and geranyl acetate from their mandibular glands as territory markers in grassy habitats [Vinson *et al.*, 1982].

High female density advances mating behaviour at female aggregation sites. On the other hand, mating behaviour at non-resource-based or resource-based sites is correlated with a low female density. In general, mating behaviour at female emergence sites is mediated by female pheromones independent of non-territorial or territorial male behaviour because females announce by the release of a pheromone that they are ready for mating. Oligolectic female bees use only a limited range of flowers for feeding and location of females is more straightforward for males. Pheromone production is needless in these cases. On the other hand, polylectic female bees are all-around and pheromones are essential for mating success. Male and female produced pheromones are involved in non-territorial male mating behaviour in these cases. On the other hand, territorial male mating relies mainly on male-derived pheromones.

Mating in *A. compressa* and *L. niger* takes place at female emergence sites and thus, if pheromones are present, it is expected that these are female-derived.

2.4 Analysis of cuticular waxes of female and male *Ampulex compressa* and those of the female Dufour gland

The composition and the relative abundance of each compound in the extracts is shown in Table 1. The comparison of total ion chromatograms (TICs) of the extracts in Figure 5 showed clearly that the lipid profiles of female cuticle and DG overlapped qualitatively, whereas the lipid profile of the male cuticle differed from both. Obviously, a sexual dimorphism in the hydrocarbon profile exists. The most striking features of the hydrocarbon profiles are now briefly discussed, followed by a description of the structure elucidation of interesting compounds.

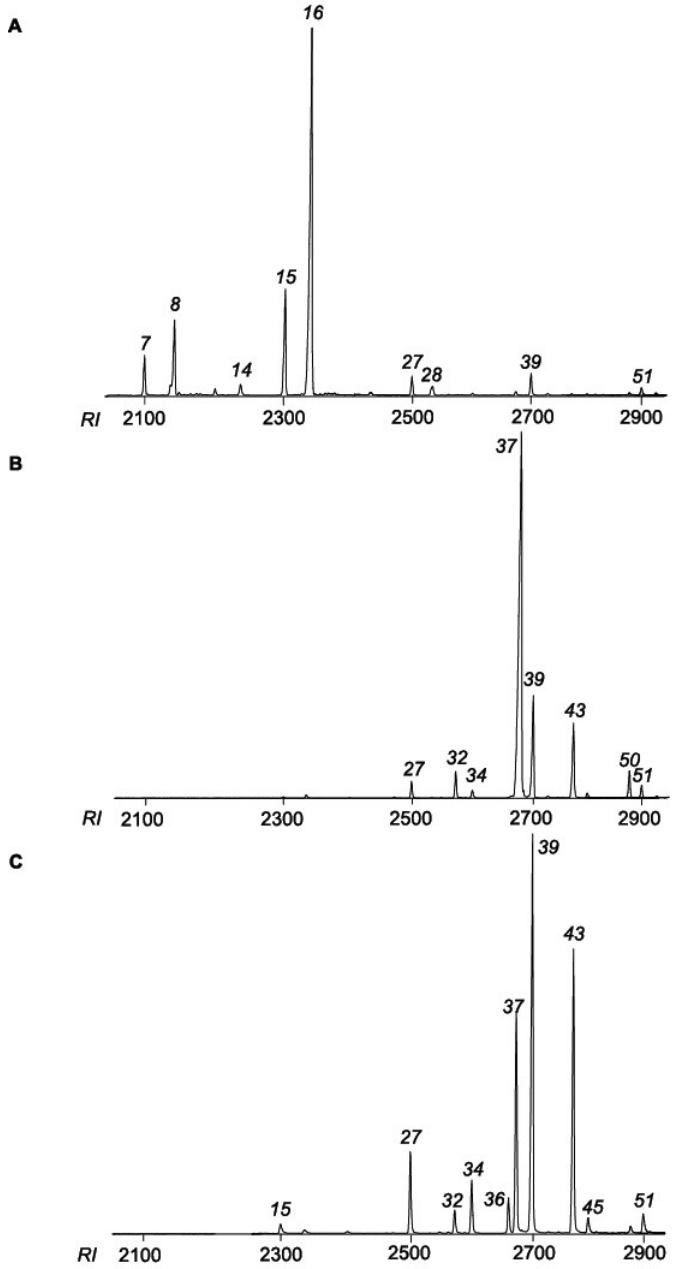

Figure 5: Total ion chromatograms of **(A)** male cuticule extract, **(B)** female cuticle extract, **(C)** extract of the Dufour gland. Numbers refer to the compounds in Table 1.

Table 1: Identified compounds of *Ampulex compressa* in %. Male, Female: lipid composition of male and female cuticle; DG: lipid composition of the Dufour gland. Position of double bonds were determined by derivatization with dimethyl disulfide (DMDS). X = position undetermined; A: artifact; [a] = Compound was detected in all investigated samples, but the content varied; [b]ratio 1:4:1; [c]ratio 5:1:5:1.

No.	*RI*	Compound	Male	Female	DG
		2-Methyl-3-pentanone	-	-	X[a]
		3Z-9:al	Trace	-	-
1	1800	18:H	Trace	-	-
2	1900	19:H	Trace	-	-
3	1942	9Me-19:H	Trace	-	-
	1942	7Me-19:H	„	-	-
4	2000	20:H	Trace	-	-
5	2033	18:al	Trace	-	-
6	2040	6Me-20:H	0.1	-	-
	2040	7Me-20:H	„	-	-
7	2100	21:H	4.0	0.4	Trace
8	2144	9Me-21:H	9.0	0.6	-
	2144	7Me-21:H	„	„	-
	2144	5Me-21:H	„	-	Trace
9	2173	7,13DiMe-21:H	0.1	-	-
10	2178	7,15DiMe-21:H	0.1	-	-
11	2186	5,15DiMe-21:H	Trace	-	-
12	2200	22:H	0.6	Trace	Trace
13	2209	3,15DiMe-21:H	0.1	-	-
14	2235	8Me-22:H	1.7	0.1	-
	2235	9Me-22:H	„	„	-
15	2300	23:H	14.2	0.7	1.1
16	2341	7Me-23:H	-	2.7	-
	2341	9Me-23:H	53.5	„	Trace
17	2365	9,13DiMe-23:H	0.1	-	-
18	2369	9,15DiMe-23:H	0.2	-	-
19	2374	7,15DiMe-23:H	0.2	-	-
20	2378	5,9DiMe-23:H	0.2	-	-
21	2382	5,15DiMe-23:H	0.1	-	-
22	2400	24:H	0.3	Trace	Trace
23	2406	3,15DiMe-23:H	0.1	-	-
24	2441	9Me-24:H	0.5	Trace	-
	2441	10Me-24:H	„	„	-
25	2470	X,Xdiene-25:H	Trace	0.2	-
26[b]	2491	6ene-25:H	-	0.1	-
	2491	7ene-25:H	-	„	-
	2491	9ene-25:H	-	„	-
27	2500	25:H	2.8	2.1	6.7
28	2536	9Me-25:H	2.3	0.2	-
	2536	11Me-25:H	„	„	-
29	2547	Phthalate (A)	0.1	0.1	-
30	2559	9,13DiMe-25:H	0.1	-	-

No.	*RI*	Compound	Male	Female	DG
31	2563	2Me-25:H	-	-	Trace
32	2571	3Me-25:H	0.1	4.1	2.1
33[c]	2584	6ene-26:H	Trace	0.1	-
	2584	7ene-26:H	„	„	-
	2584	8ene-26:H	„	„	-
	2584	9ene-26:H	„	„	-
34	2600	26:H	0.2	0.9	4.3
35	2629	10Me-26:H	0.1	-	-
	2629	11Me-26:H	„	-	-
	2629	12Me-26:H	„	-	-
36	2658	2Me-26:H	0.1	-	2.9
37	2670	6*Z*,9*Z*-27:H	0.6	66.2	18.7
38	2693	7ene-27:H	-	Trace	-
	2693	9ene-27:H	-	„	-
39	2700	27:H	3.7	6.9	36.2
40	2726	9Me-27:H	0.4	-	-
	2726	11Me-27:H	„	0.4	-
	2726	13Me-27:H	„	„	-
41	2744	5Me-27:H	-	0.1	-
42	2755	9,13DiMe-27:H	0.1	-	-
43	2770	3Me-27:H	0.3	9.3	23.6
44	2785	6,9diene-28:H	-	Trace	-
45	2800	28:H	0.2	0.5	1.5
46	2815	Squalene	-	0.1	-
47	2828	12Me-28:H	0.1	-	-
	2828	13Me-28:H	„	-	-
	2828	14Me-28:H	„	-	-
48	2847	unknown	Trace	-	-
49	2852	Octadecyl benzoate	0.1	-	-
50	2877	6,9diene-29:H	-	2.7	0.7
	2877	9ene-29:H	0.4	-	-
51	2900	29:H	1.7	0.8	2.2
52	2928	11Me-29:H	-	0.2	-
	2928	13Me-29:H	0.6	„	-
	2928	15Me-29:H	„	„	-
53	2947	9,13DiMe-29:H	0.1	-	-
	2947	11,15DiMe-29:H	„	0.1	-
	2947	13,17DiMe-29:H	„	„	-
54	2973	3Me-29:H	-	0.2	Trace
55	2973	Xene-30:H	Trace	-	-
56	3000	30:H	Trace	0.1	-
57	3028	13Me-30:H	Trace	-	-
	3028	14Me-30:H	„	-	-
	3028	15Me-30:H	„	-	-
58	3064	Eicosyl benzoate	Trace	-	-
59	3080	X,Xdiene-31:H	-	Trace	-
60	3080	Xene-31:H	0.2	Trace	-
61	3100	31:H	0.1	0.1	-
62	3120	XMe-nonacosan-10-one	Trace	-	-

No.	*RI*	Compound	Male	Female	DG
63	3127	11Me-31:H	-	Trace	-
	3127	13Me-31:H	-	„	-
	3127	15Me-31:H	0.3	„	-
64	3150	15,19DiMe-31:H	Trace	-	-
65	3225	XMe-triacontan-10-one	Trace	-	-
66	3275	Docosyl benzoate	0.2	-	-
67	3327	17Me-hentriacontan-10-one	0.2	-	-

The most abundant compound class in the DG with 52.2% were *n*-alkanes, followed by monomethyl branched alkanes (28.9%) and alkadienes (19.4%). Heptacosane (27:H) with 36.2% was the most abundant *n*-alkane whose chain length ranged from 21 to 29 carbon atoms. The monomethyl branched alkanes (21-27 carbon atoms) were dominated by 3-methylheptacosane (3Me-27:H, 23.6%). The position of the methyl groups in these derivatives was deduced by calculation of retention indexes (*RI*s) and the observation of mass spectrometric fragmentation patterns. Calculation of *RIs* allows predictions about the branching position in the chain because the dependence of the *RI* from the position of the methyl group in the chain is well-known [Carlson *et al.*, 1998; Schulz, 2001]. The mass spectra of monomethyl branched hydrocarbons show characteristic α-cleavages at the branching point, e. g. m/z = 365 in the case of 3Me-27:H [Szafranek *et al.*, 1994]. Its *RI* was determined as 2770 and this value was closed to the predicted one of 2773. The theoretical value RI_{theo} was calculated by summing up 2700 points for the alkyl chain and 73 points for the methyl group in (ω-2)-position. By definition, the *RI* of a *n*-alkane corresponds to the number of carbon atoms multiplied with hundred [van den Dool and Kratz, 1963].

The blend of alkadienes in the DG comprised (6*Z*,9*Z*)-6,9-heptacosadiene (6*Z*,9*Z*-27:H, 18.7%) and 6,9-nonacosadiene. The double bond positions were deduced by GC-MS-analysis of the corresponding DMDS adducts after derivatization with dimethyl disulfide (DMDS) [Carballeira and Cruz, 1996].

The most abundant compound of the female cuticle was (6*Z*,9*Z*)-27:H with 65.7%. Alkadienes contributed 68.7% to the TIC of this extract, followed by *n*-alkanes (17.2%) and monomethyl alkanes (12.9%). The chain length of the *n*-alkanes ranged from 21 to 31 carbon atoms with 27:H (6.9%) as the most important representative. The monomethyl alkanes varied between 21 and 29 carbon atoms and a methyl branch in position 3 was preferred (3Me-27:H, 9.3%).

In general, males formed hydrocarbons with a lower molecular weight than females. Unbranched alkanes contributed 27.8% to the composition of the male lipids and the chain length ranged from

18 to 31 carbon atoms. Tricosane was the most abundant representative of this compound class (14.2%) as well as the second major compound in the total blend. Monomethyl alkanes between 19 and 31 carbon dominated the lipid blend (68.3%) with 9-methyltricosane (9Me-23:H, 52.9%) as most prominent compound. The branching pattern of this compound was elucidated by the intensive ions *m*/*z* = 140/141 and 224/225 in its mass spectrum as shown in Figure 6.

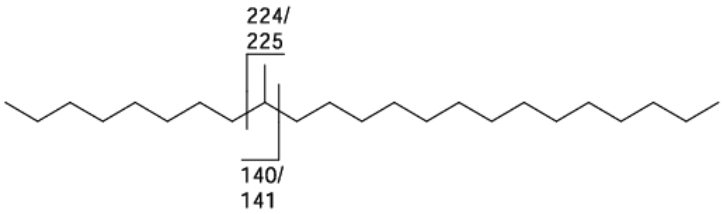

Figure 6: Fragmentation pattern for monomethyl alkanes exemplified for 9-methyltricosane.

Normally, methyl groups occurred in odd-numbered positions if the chain length was also odd-numbered. In contrast, if the chain-length was even-numbered, then methyl groups were either in odd-numbered or in even-numbered positions. Minor compounds were dimethyl alkanes and their chain length spanned 21 - 29 carbon atoms. This compound class contributed 1.6 % to the TIC and methyl branches occurred predominantly internally on odd-numbered carbon atoms. The branching patterns of these derivatives were also elucidated by thorough analysis of their mass spectra. In the case of 9,13DiMe-23:H, the characteristic ions *m*/*z* = 140/141, 168/169, 211, and 239 proved this arrangement of the methyl groups caused by the above mentioned fragmentations (Figure 7) [Szafranek *et al.*, 1994].

Alkenes (0.6 %) and alkadienes (0.6%) occurred only in subordinate amounts.

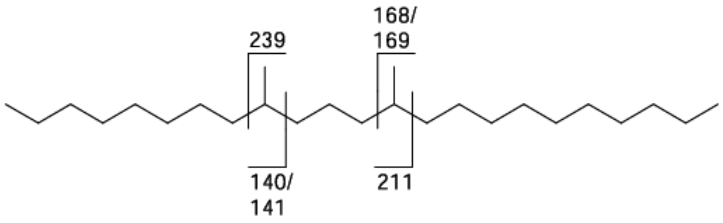

Figure 7: Fragmentation pattern for dimethyl alkanes exemplified for 9,13-dimethyltricosane.

Finally, the biosynthesis of insect alkanes is briefly discussed [Morgan, 2004]. Odd-numbered alkanes start the biosynthesis with an acetate unit. Then the chain is extended by the addition of acetate via malonate to an appropriate length followed by reduction to an aldehyde. Finally, this aldehyde is decarboxylated in the presence of oxygen, cytochrome P_{450} and $NADP^+$ [Reed *et al.*, 1994; Reed *et al.*, 1995]. In contrast, even-numbered alkanes use propionate as starter

unit followed by the above mentioned steps to provide the alkane. In insects, acetate is preferentially used as starter unit and thus odd-numbered alkanes dominate in the corresponding lipid blends. The application of propionate via methyl-malonate during chain extension furnishes methyl branched alkanes and in the case of odd-numbered derivatives, methyl groups are attached to odd-numbered carbon atoms. On the other hand, even-numbered methyl branched alkanes bear the methyl group either on odd- or on even-numbered carbon atoms. This pattern can have two origins: 1) If propionate is used as starter unit, then methyl groups occur in even-numbered positions. 2) If propionate is used as final unit during chain extension, then methyl groups are in odd-numbered positions. The biosynthesis of hydrocarbons proceeds in oenocytes, located in the epidermis, independent of their local requirement. The hydrocarbons are transported through the hemolymph to their different domains by a special transport protein, called lipophorin [Schal *et al.*, 1998a].

2.4.1 Structure elucidation of (6Z,9Z)-6,9-Heptacosadiene

This compound, showing a *RI* of 2670 eluted shortly before 27:H and was characterized by a molecular weight of 376 amu. The mass spectrum of this compound is drawn in Figure 8.

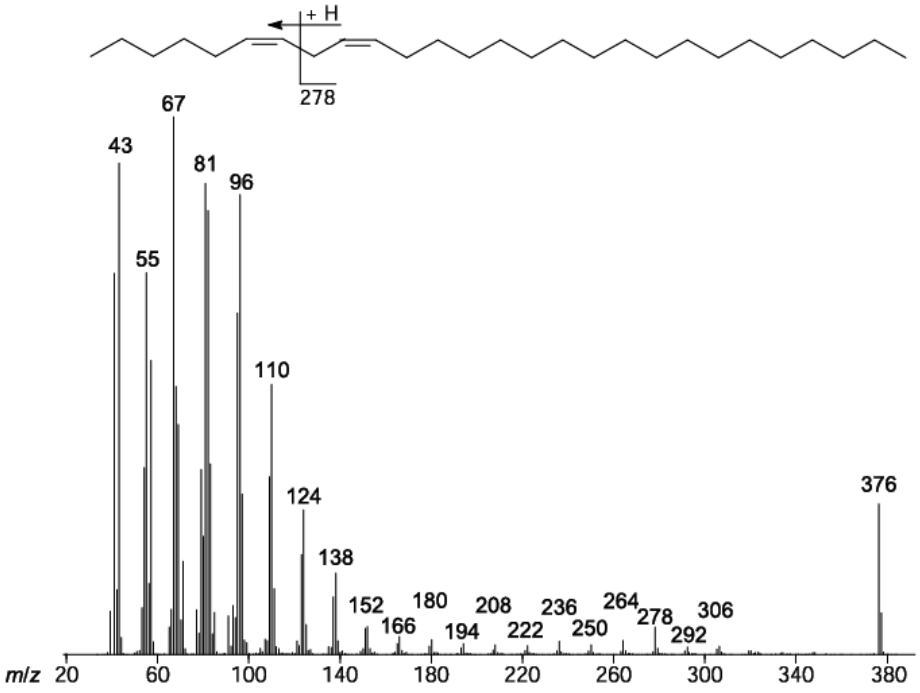

Figure 8: Mass spectrum of (6Z,9Z)-6,9-heptacosadiene and fragmentation leading to the ion $m/z = 278$.

The mass spectrum of this compound resembled those of alkadienes. The diene structure was indicated by the ions $m/z = 67$ and 81 and the ion series with the general formula C_nH_{2n-2}. This series

is characteristic for dienes and therein the ions *m*/*z* = 96 and 110 were prominent. The occurrence of the ion *m*/*z* = 278 ($[M - 98]^+$) led to the assumption that a 6,9-arrangement of the double bonds existed because this kind of fragmentation was reported to be characteristic for these dienes and these ions are thought to be formed by a cleavage between carbon atom 7 and 8 with a final hydrogen transfer to the shorter fragment [Descoins *et al.*, 1986].

This proposal was confirmed by treating the natural extract with DMDS and subsequent interpretation of the mass spectra of the corresponding DMDS-adducts of (6Z,9Z)-27:H. In general, structures according to Figure 9 are proposed for DMDS-adducts of dienes with a methylene interrupted double bond system [Carballeira and Cruz, 1996].

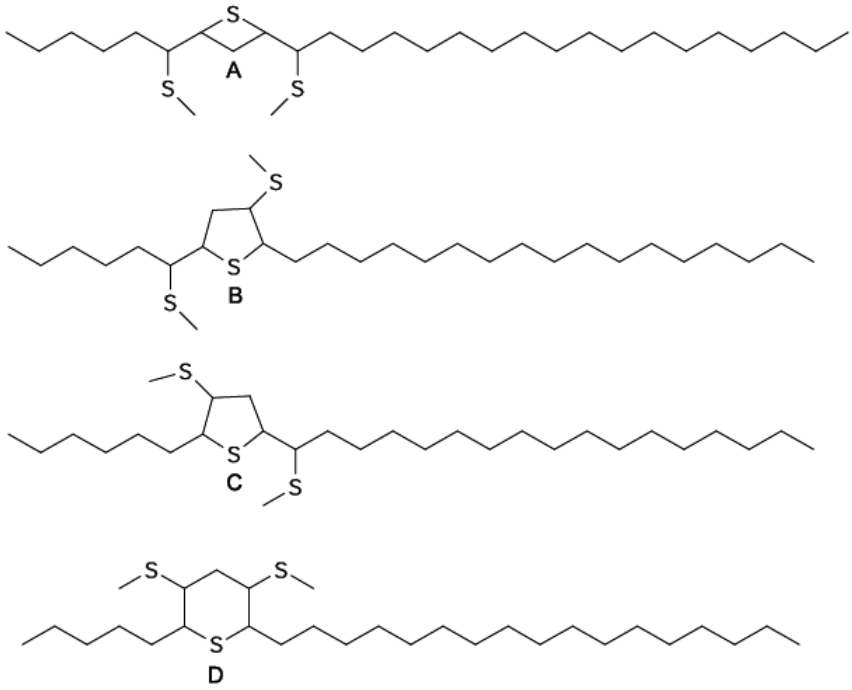

Figure 9: Corresponding postulated structures for DMDS-adducts (502 amu) of (6Z,9Z)-6,9-heptacosadiene.

During this analysis, four DMDS-adducts with the expected molecular weight of 502 amu were obtained and these derivatives formed two pairs of compounds with different mass spectra. The appearance of DMDS-adducts with identical mass spectra was expected because four stereogenic centers are *anti*-selectively generated by this derivatization leading to two diastereoisomers.

The mass spectra of the first stereoisomeric pair were characterized by the ions *m*/*z* = 155, 203, and 299 whereas the mass spectra of the second stereoisomeric pair exhibited abundant ions at *m*/*z* = 131, 323, and 371 as illustrated in Figure 10. The full scan mass spectra of these compounds are depicted in Figure 11 and 12. Both mass spectra contained also the ions

m/*z* = 407 and 454. The mass spectra were in accordance with the derivatives B and C in Figure 9.
The ions *m*/*z* = 131 and 371 were formed by a cleavage between the first carbon atom of the side chain bearing the thiomethyl group and the neighboured ring carbon atom. Furthermore, elimination of thiomethanol converted the ion *m*/*z* = 371 into the ion *m*/*z* = 323. Elimination of thiomethanol is favoured because a resonance stabilized ion is formed by this fragmentation as illustrated in Figure 13. First, **1** fragments in α-position to the ring in direction to the 1-thiomethylalkyl chain under formation of **2**. An elimination of thiomethanol furnishes the stabilized ion **3** which is in resonance to the ions **4** and **5**.

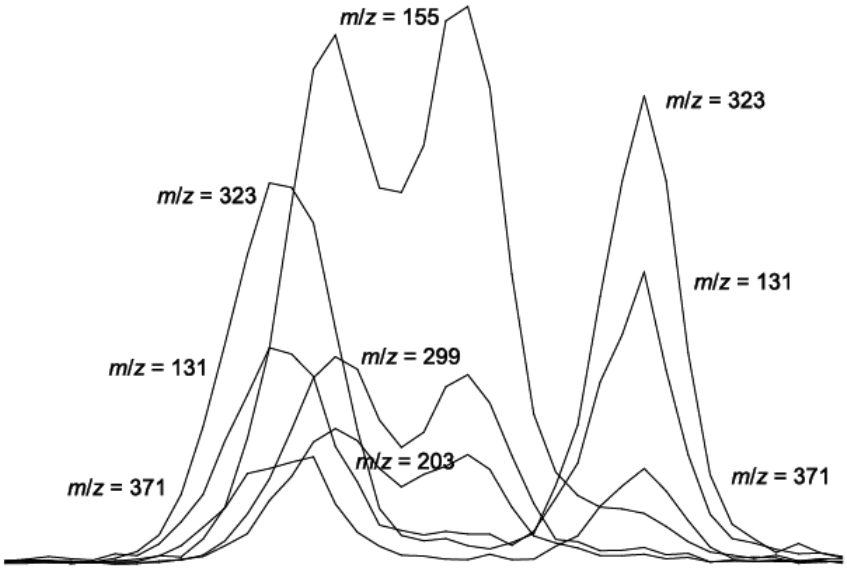

Figure 10: Ion traces of DMDS-adducts with the molecular weight of 502 amu in dependence of the ions *m*/*z* = 131, 155, 203, 299, 323, and 371.

Analogous fragmentations in the other isomer furnished the ions *m*/*z* = 155, 203, and 299.
Based on these mass spectral interpretations, a 6,9-arrangement of the double bonds was concluded. Generally, natural dienes with this double bond arrangement are characterized by an all-*cis*-configuration of the double bond system [Krokos *et al.*, 2001].
Hence, 6*Z*,9*Z*-27:H was synthesized according to Figure 14 to prove this assumption. Commercial available methyl linoleate **6** was used as starting compound. Reduction with lithium aluminium hydride (LAH) provided the alcohol **7** in 86% yield. Then, **7** was brominated with triphenylphosphane and bromine to furnish compound **8** in 55% yield [Sonnet, 1976]. Target compound **9** was finally prepared in 14% yield by coupling of **8** and *n*-nonylmagnesium bromide in the presence of Li_2CuCl_4 [Tamura and Kochi, 1971]. The turnover was quantitative in this reaction but the yield was low because octacosane (18:H) was formed as side product. Chromatographic

properties of both compounds were similar and coelution took partly place during purification by silica gel chromatography with pentane as solvent. The formation of 18:H proceeded during the preparation of *n*-nonylmagnesium bromide. Coinjection of the synthetic sample and natural extract proved the (6*Z*,9*Z*)-arrangement of the double bonds in the natural compound.

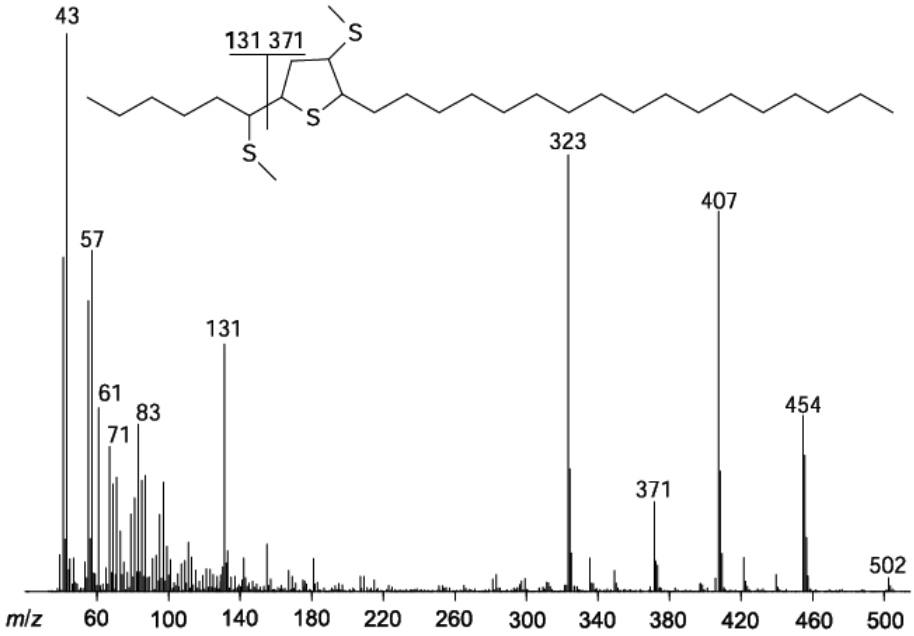

Figure 11: Mass spectrum of the derivative B (Figure 9).

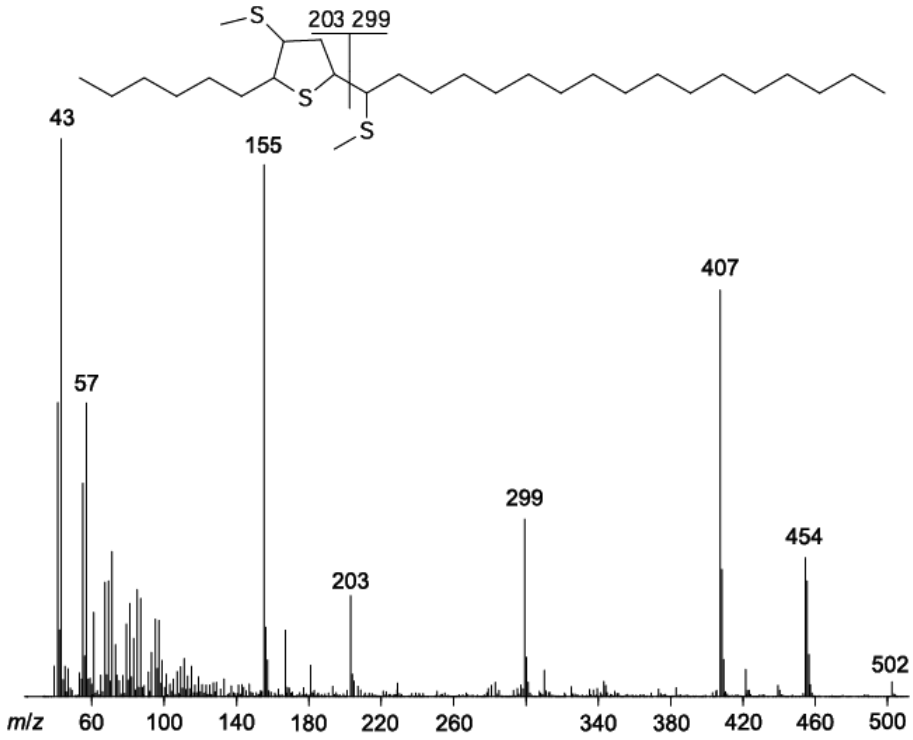

Figure 12: Mass spectrum of the derivative C (Figure 9).

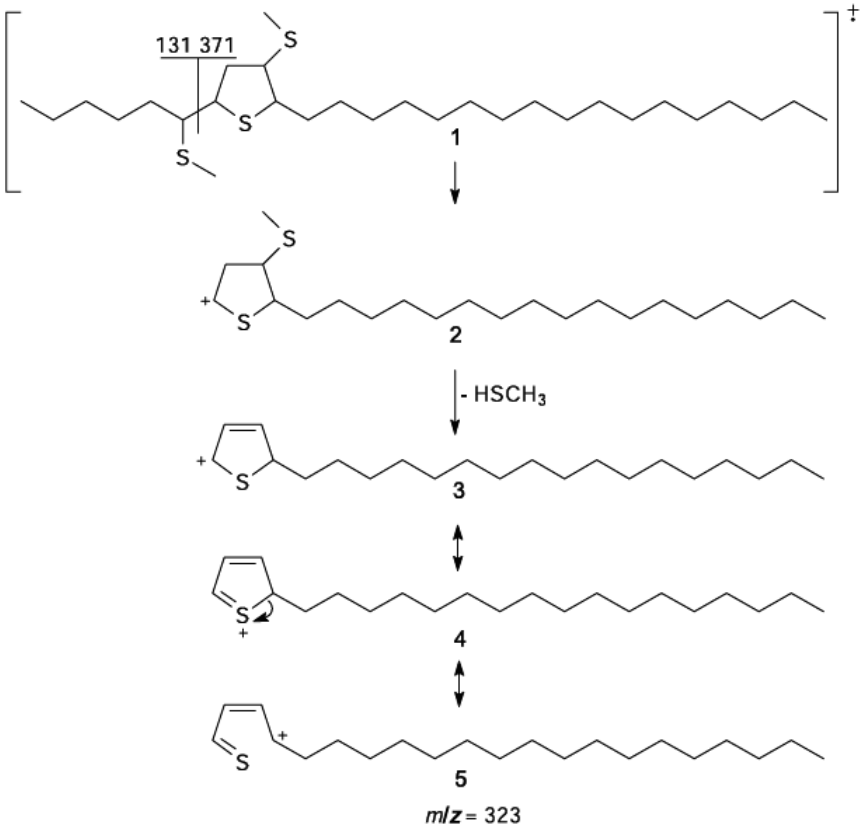

Figure 13: Proposed fragmentation pattern for a DMDS-adduct of (6Z,9Z)-6,9-heptacosadiene.

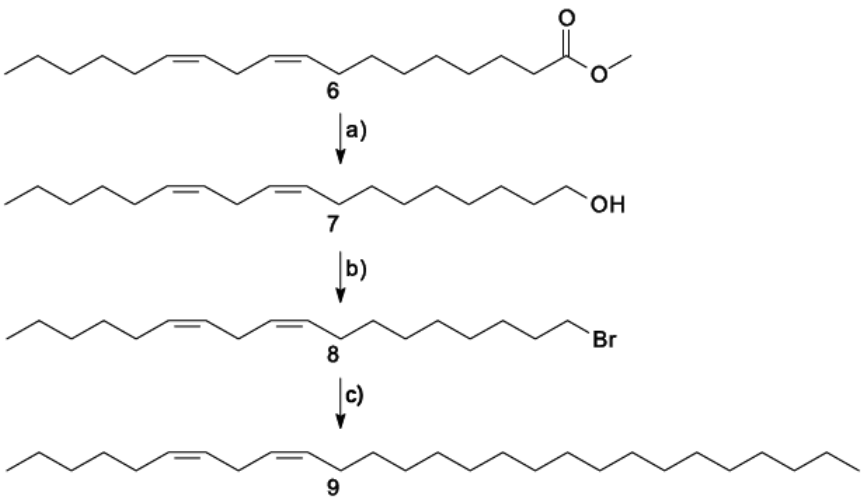

Figure 14: Synthesis of (6Z,9Z)-6,9-heptacosadiene. a) anhydr. Et_2O, $LiAlH_4$, 16h rt, 86%; b) anhydr. CH_2Cl_2, PPh_3, Br_2, 16h rt, 55%; c) anhydr. THF, Li_2CuCl_4, $C_9H_{19}MgBr$, -78°C to rt, 14%.

Furthermore, this arrangement of the double bonds was also confirmed for the nonacosadiene by the corresponding DMDS-adducts (530 amu). These derivatives were characterized by the ions *m*/*z* = 155, 203, and 327 as well as *m*/*z* = 131, 351, and 399.

This compound and other dienes are most likely biosynthetically formed according to Figure 15. First, octadecanoic acid (18:COOH) **10** is desaturated by a Δ9-desaturase to form oleic acid (9*Z*-18:COOH) **11**. In general, all organism are capable to introduce a desaturation in this position. A further desaturation by a Δ12-desaturase furnishes linoleic acid (9*Z*,12*Z*-18:COOH) **12**. Animals are usually unable to perform this step but plants and fungi can perform this conversion [Dewick, 2006]. This assumption was disproved by investigations about linoleic acid biosynthesis in the cockroach *Periplaneta americana*, in the cricket *Acheta domesticus*, and the termite *Zootermopsis angusticollis* [Blomquist *et al.*, 1982]. Later, de novo biosynthesis of linoleic acid was also proved in the pea aphid *Acyrthosiphon pisum* [de Renobales *et al.*, 1986]. Application of radiolabeled acetate and oleic acid provided radiolabeled linoleic acid and thus, this organism contains a Δ12-desaturase essential for this transformation. Hence, de novo biosynthesis of linoleic acid or its dietary uptake have to be regarded for the biosynthesis of 6*Z*,9*Z*-dienes **14**. The addition of acetate units via malonate provides the chain-elongated intermediates **13** which are finally converted to **14** after reduction of **13** to the corresponding aldehydes and subsequent decarboxylation.

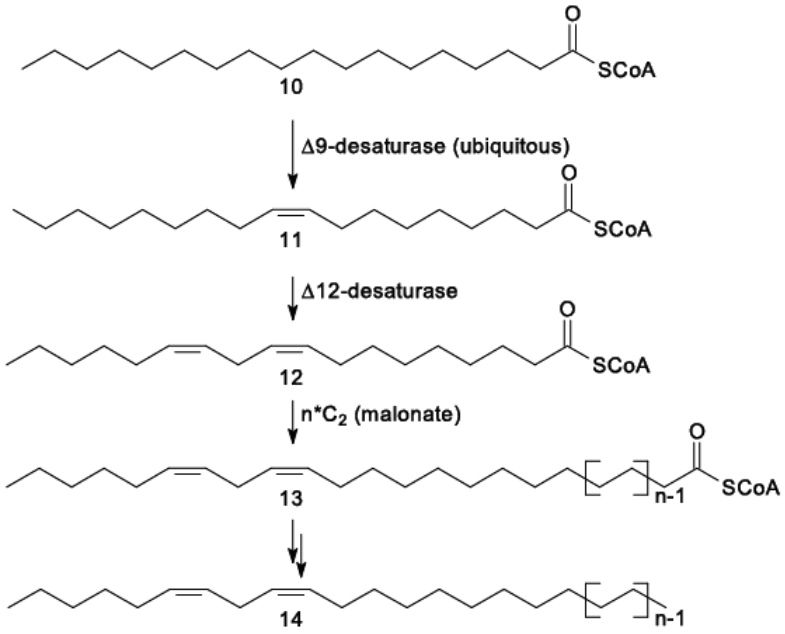

Figure 15: Proposed biosynthesis of (6*Z*,9*Z*)-6,9-heptacosadiene.

2.4.2 Determination of double-bond positions in alkenes of *Ampulex compressa*

Long-chain alkenes were present in the lipid profile of the male cuticle, female cuticle and DG. The double bond positions were also elucidated by derivatization with DMDS [Scribe *et al.*, 1990]. Mass spectra of DMDS-adducts of monoenes are characterized by a preferred cleavage between the carbon atoms bearing the thiomethyl moieties as shown for the DMDS-adduct of 7-heptacosene in

Figure 16.

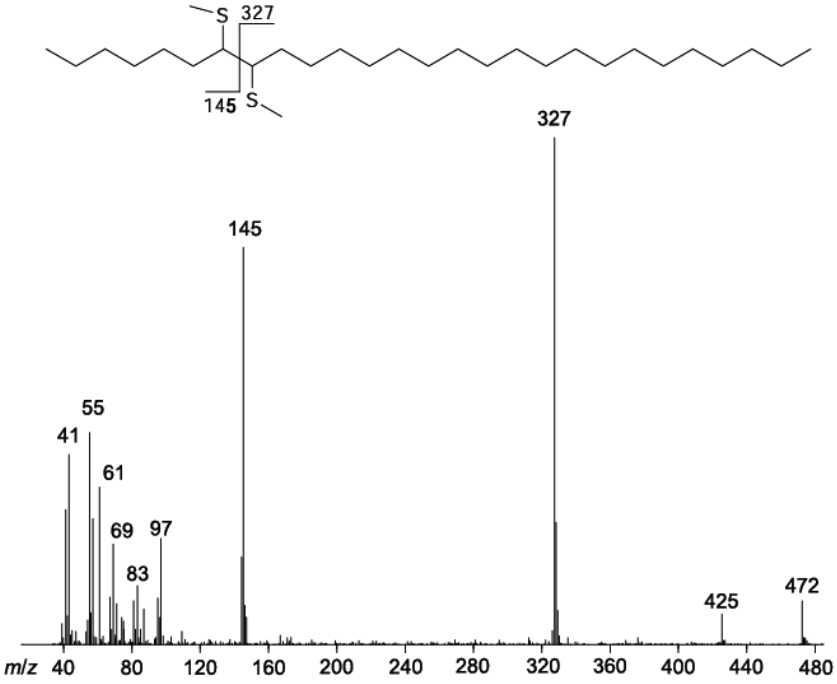

Figure 16: Mass spectrum of the DMDS-adduct of 7-heptacosene.

The ion *m*/*z* = 472 indictated the molecular ion of the DMDS-adduct as expected for an alkene with 27 carbon atoms. The ions *m*/*z* = 145 and 327 represented the most prominent ions of this mass spectrum and by knowledge of the fragmentation pattern, these pointed to a desaturation in position 7. Based on this method, the double bond position of other alkenes was ascertained (Table 2).

Table 2: Identified alkenes of *Ampulex compressa*. The key ions correspond to the double bond determing fragments in their mass spectra. Intensities of alkenes are given in %.

Compound	key ions	Male	Female	DG
5ene-25:H	117; 327	-	Trace	-
6ene-25:H	131; 313	-	Trace	-
7ene-25:H	145; 299	-	2	Trace
9ene-25:H	173; 271	-	Trace	-
6ene-26:H	131; 327	-	6	6
7ene-26:H	145; 313	-	Trace	-
8ene-26:H	159; 299	-	5	Trace
9ene-26:H	173; 285	-	Trace	-
5ene-27:H	117; 355	-	10	14
7ene-27:H	145; 327	-	41	39
9ene-27:H	173; 299	-	36	41
6ene-28:H	131; 355	-	Trace	-
8ene-28:H	159; 327	-	Trace	-
9ene-29:H	173; 327	100	-	-

Alkenes were rare in the lipid blend of the male cuticle and only 9ene-29:H was present. In contrast, the alkene blend of the female cuticle and the DG was more diverse and these blends were dominated by 7ene-27:H and 9ene-27:H. Double bonds occurred in 5-, 6-, 7-, 8- and 9-position.

2.4.3 Presence of 2-methyl-3-pentanone in the Dufour gland of *Ampulex compressa* females

Besides the long-chain hydrocarbons, a single volatile compound, 2-methyl-3-pentanone, was present in every investigated DG extract and its mass spectrum is shown in Figure 17. This structure was proposed because of identical mass spectra of natural compound and synthetic reference.

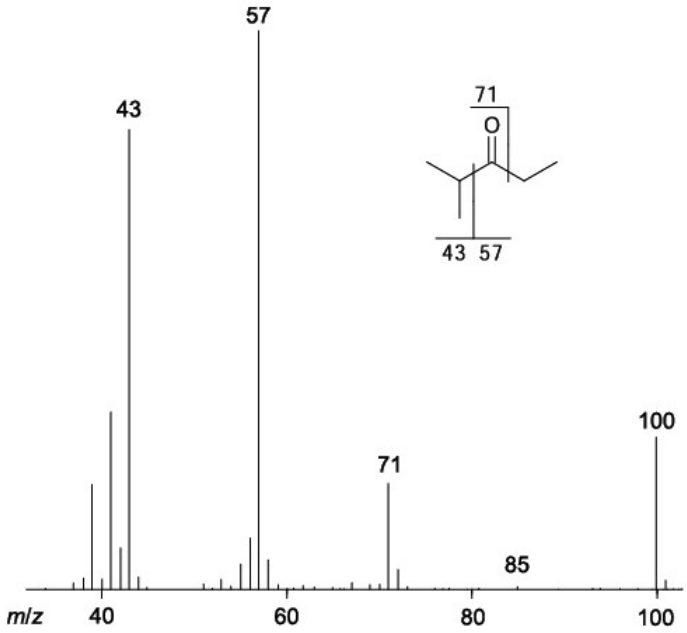

Figure 17: Mass spectrum of 2-methyl-3-pentanone.

Similar branched short-chain ketones play a role in the semiochemistry of other hymenoptera. 3-Methyl-2-pentanone is part of the pheromone blend of the african ant *Crematogaster nigriceps* [Wood *et al.*, 2002]. Besides, the homologous compound was reported to act as pheromone in the ant *Pseudomyrmex nigrocinta* [Wood, 2005]. Another branched short-chain ketone, 4-methyl-2-pentanone, is a kairomone and support the location of the fox *Vulpes vulpes* by the sandfly *Lutzomyia longipalpis* [Dougherty *et al.*, 1999].

According to the fact that similar branched short-chain ketones were described as pheromones in hymenopteren species, this compound deserves to be tested in bioassays to prove a possible function in the semiochemistry of A. *compressa*.

A biosynthetic pathway, leading to 2-methyl-3-pentanone **19**, is proposed in Figure 18.

First, (*S*)-valine **15** is transaminated to the α-keto acid **16** by the enzyme transaminase in the presence of glutamic acid and pyridoxal phosphate (PLP). Then α-oxidation takes place under

formation of acid **17** requiring thiamine diphosphate (TPP), lipoic acid and coenzyme A followed by chain extension via methylmalonate to furnish **18**. Finally, decarboxylation provides **19**.

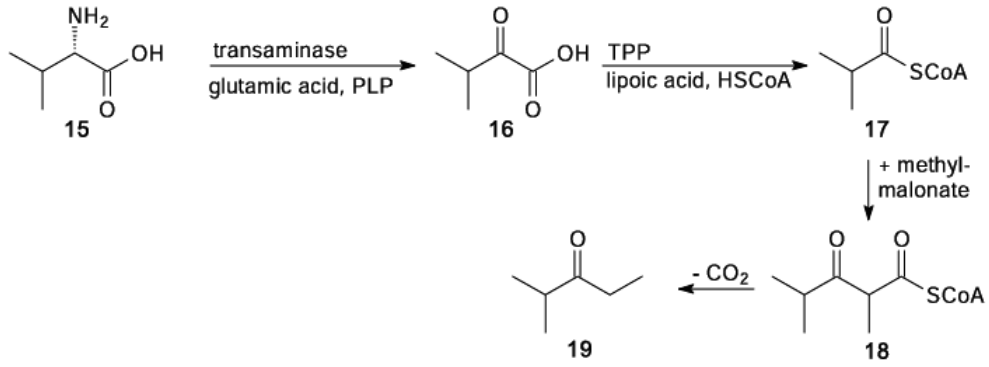

Figure 18: Proposed biosynthesis of 2-methyl-3-pentanone [Dewick, 2006].

2.4.4 (*Z*)-3-Nonenal, an unusual aldehyde on the cuticle of male *Ampulex compressa*

An unusal unsaturated aldehyde, (*Z*)-3-nonenal (3*Z*-9:al), was identified on the cuticle of *A. compressa* males. Its *RI* was 1104 and the corresponding mass spectrum is depicted in Figure 19.

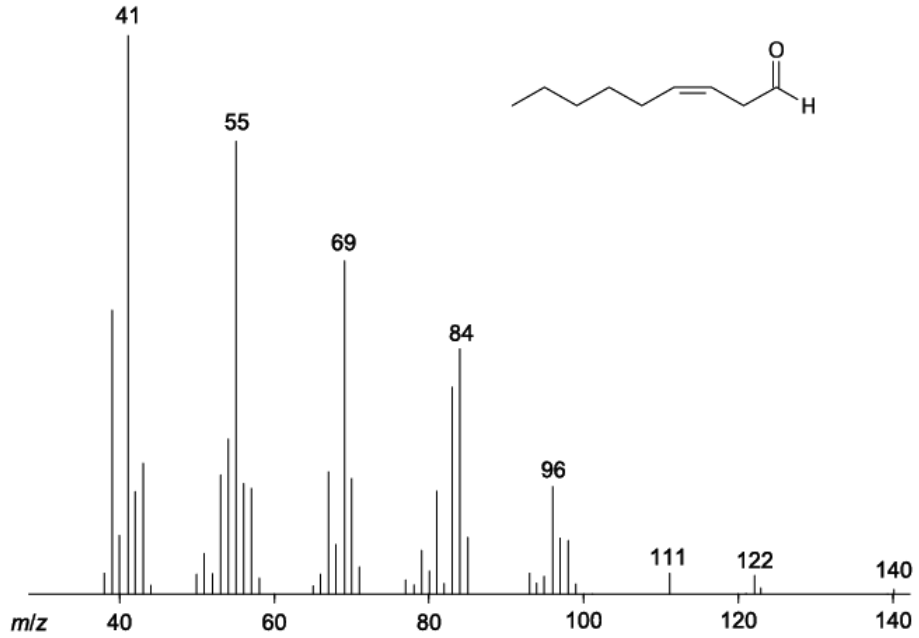

Figure 19: Mass spectrum of (*Z*)-3-nonenal.

This compound eluted shortly before nonanal (9:al, *RI* = 1116). The mass spectrum of 9:al was characterized by the molecular ion *m*/*z* = 142, the ions *m*/*z* = 114, and the ion *m*/*z* = 124. The latter ones are typical for saturated aldehydes and the cleavage of H_2O and C_2H_4 are responsible for their formation [Budzikiewicz, 1967]. In contrast, the mass spectrum of the unknown compound showed the ions *m*/*z* = 122 and *m*/*z* = 140. The latter one was the molecular ion which lost H_2O to form the ion *m*/*z* = 122. According to these observations, an unsaturated aldehyde was proposed as structure.

A double bond in position 2 was ruled out because the fragmentation pattern and also the *RI* of the natural compound deviated from those of synthetic references. Both isomers with the double bond in position 2 elute after 9:al on a BPX-5-phase. The mass spectrum of the natural compound was very similar to a reference mass spectrum of (3*Z*)-9:al [Sakuma and Kowaka, 1994]. Final proof for (3*Z*)-9:al **20** as structure was obtained by oxidation of (*Z*)-3-nonenol with an excess of pyridinium chlorochromate (PCC) in anhydr. CH_2Cl_2 in 72 % yield [Barker *et al.*, 1989]. The mass spectrum of **20** and its *RI* matched those of the natural product.

This aldehyde is unstable and rapidly converted into 4-hydroxy-(2*E*)-nonenal in the presence of air in a nonenzymatic process or an isomerisation to (*E*)-2-nonenal takes place [Galliard and Philipps, 1976; Noordermeer *et al.*, 2001] However, no degradation products were detected in the extracts and it may be that, antioxidants in the cuticle prevent the degradation of this compound.

Previously, **20** was identified as a volatile in stored beer bottles [Barker *et al.*, 1989]. It occurs in cucumber and in other plants, e. g. in the moss *Physcomitrella patens* as well as in the brown alga *Laminaria angustata*. [Gargouri and Legoy, 1998; Boonprab *et al.*, 2006; Stumpe *et al.*, 2006]. The occurrence of (3*Z*)-9:al as natural product in insects was unknown so far.

In cucumber, the biosynthesis of **20** and other aldehydes is induced after mechanical wounding; furthermore, these compounds possess fungicidal activity [Matsui *et al.*, 2006]. Experiments with transgenic potato plants lacking a hydroperoxide lyase showed that this enzyme is essential for the formation of **20** [Vancanneyt *et al.*, 2001]. Such plants were more attractive for aphids than the wild-type demonstrating a potential repellent activtiy of this compound against insects.

2.4.4.1 Biosynthesis of (*Z*)-3-nonenal in plants

Polyunsaturated acids serve as precursors for 3*Z*-9:al and other aldehydes in plants and other organisms [Schneider *et al.*, 2001]. A biosynthetic scheme leading to **20** by the oxidative degradation of linoleic acid **21** and other PUFAs is illustrated in Figure 20 [Crombie and Morgan, 1991a; 1991b].

First, **21** is oxygenated by a lipoxygenase in position 9 under double bond migration to form the 10,12-diunsaturated hydroperoxide **22**. Then, the 9,10-expoxide **23** is formed by a nucleophilic attack of carbon atom 10 after cleavage of the 10,11-double bond under degradation of the peroxy moiety followed by rearrangement of the expoxide to furnish intermediate **24**. Addition of hydroxide converts **24** into the hemiacetal **25** which decomposes to the aldehydes **26** and **20**.

Other aldehydes are accessible by regiospecific lipoxygenation or lipoxygenation of other substrates than linoleic acid, for e. g. arachidonic acid [Stumpe *et al.*, 2006].

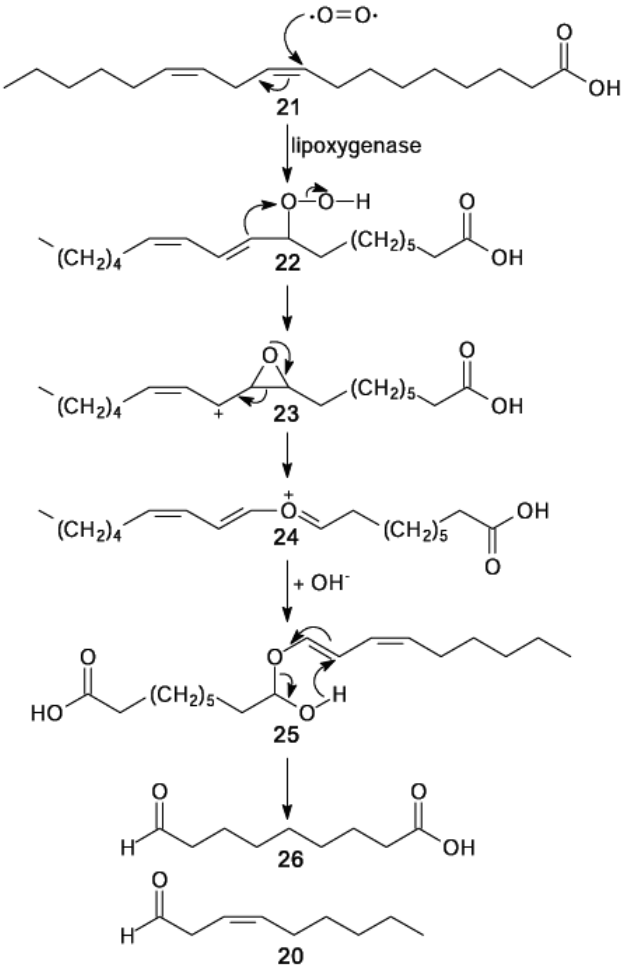

Figure 20: Proposed mechanism for the biosynthesis of 3*Z*-9:al in plants [Crombie and Morgan, 1991; 1991].

2.4.4.2 (6*Z*,9*Z*)-6,9-Heptacosadiene as precursor for the biosynthesis of (*Z*)-3-nonenal in *Ampulex compressa* males?

PUFAs as biosynthetic precursors for the biosynthesis of this compound are unlikely because they are absent in the lipid blend of the cuticle in *A. compressa* males. Alternatively, (6*Z*,9*Z*)-27:H **9** might be the precursor for the formation of this aldehyde, based on reports describing the formation of aldehydes in insects by oxidation of other compounds than PUFAs. The pheromone of the wasp *Macrocentrus grandii*, (*Z*)-4-tridecenal, is formed by oxidation of (*Z*,*Z*)-9,13-dienes in the presence of air [Swedenborg *et al.*, 1992]. An ω-oxo acetate, 9-acetoxynonanal, was reported as bioactive component in the wheat stem sawfly *Cephus cinctus* and unsaturated 1,ω-diol diacetates were postulated as precursors [Cossé *et al.*, 2002].

In an experiment to test whether **20** is formed by oxidation of **9**, a synthetic sample of **9** was subjected to UV light in the presence of air. Samples were investigated by GC-MS every 30 minutes. The formation of **20** was confirmed after 8.5 h of UV irradiation. Additional oxidation

products were identified and Figure 21 depicts the TIC of a sample of **9** after UV-irradiation for 8.5 h.

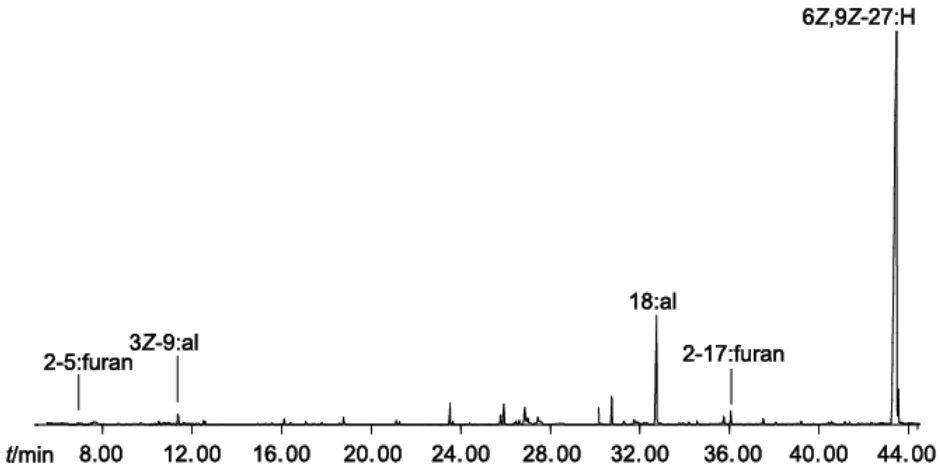

Figure 21: Total ion chromatogram after treatment of (6*Z*,9*Z*)-6,9-heptacosadiene with UV light for 8.5 h.

In addition to **20**, 18:al, 2-pentylfuran (2-5:furan) and 2-17:furan were formed in the oxidation of **9**. A pathway explaining the formation of these products is shown in Figure 22.

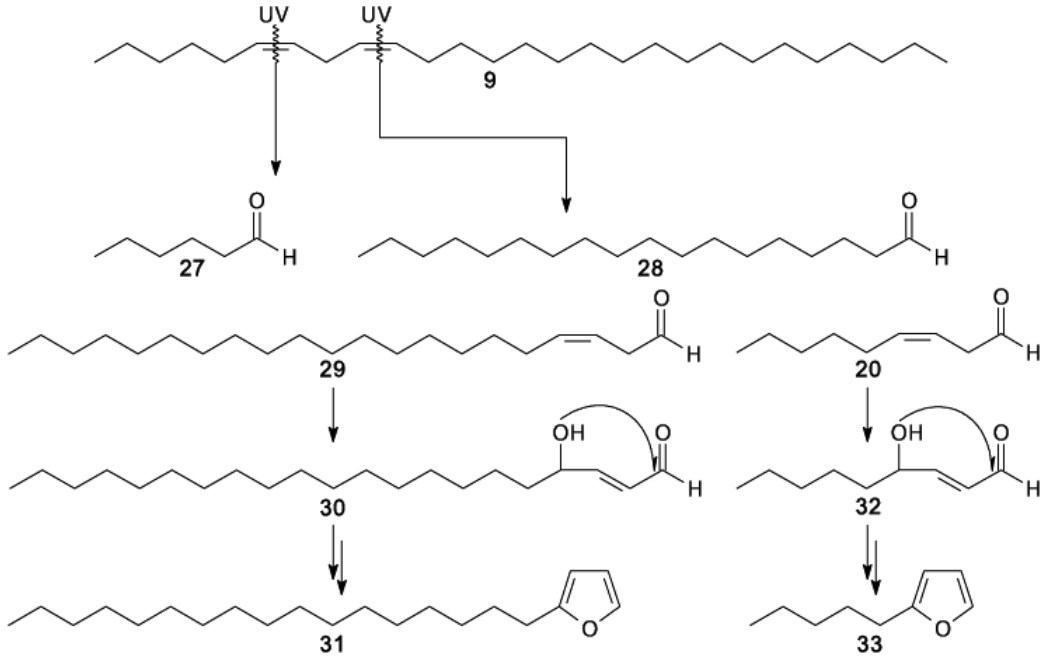

Figure 22: Proposed pathyway leading to oxidation products of (6*Z*,9*Z*)-6,9-heptacosadiene after treating for 8.5 h with UV light.

First, regiospecific lipoxygenations in position 6 and 10 followed by oxidative degradation according to Figure 20 provide hexanal **27**, octadecanal **28**, (*Z*)-3-henicosanal **29**, and **20**. The compounds **20** and **28** were identified in this experiment but **27** and **29** were absent. However, the

formation of **20** and **28** suggest also their formation. Subsequently, oxygenation of **20** and **29** in position 4 accompanied by double bond migration into the (2*E*)-configuration provide the compounds **30** and **32**. Both, **30** and **32** were not observed during this experiment, but the compounds **31** and **33** were present, indicating that **30** and **32** were involved as intermediates in their formation.

Furan **31** was prepared in a microreaction by alkylation of furan with heptadecylbromide as reference to verify its structure whereas a mass spectrum of **33** was accessible in NIST Database. The mass spectrum of **31** is shown in Figure 23, characterized by the ions *m*/*z* = 81, 95, and 306. The latter one was the molecular ion. The formation of the base ion *m*/*z* = 81 can be explained by an cleavage in allylic position to the cyclic enolether (furan) moiety. The second ion is formed by a fragmentation between carbon atoms 2 and 3 in the alkyl side chain.

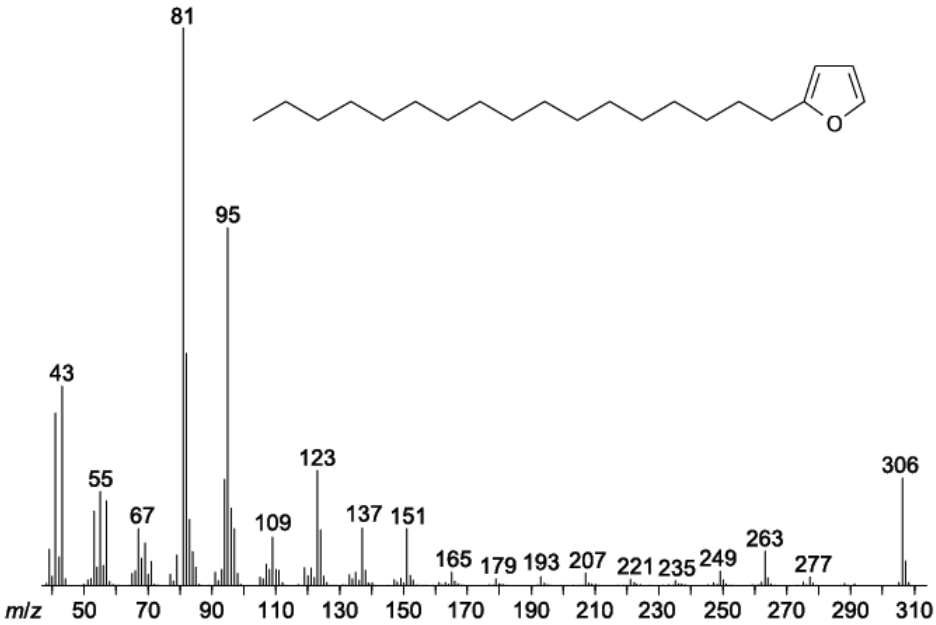

Figure 23: Mass spectrum of 2-heptadecylfuran.

Whether this oxidation process also occurs on the cuticle of *A. compressa* males remains to be elucidated.

2.4.5 Long-chain alkyl benzoates in *Ampulex compressa* males

Alkyl benzoates are rare natural compounds and so far they are unknown from insects. Pentyl benzoate and hexyl benzoate were described as flavor components in the sapodilla fruit *Manikara zapota*, a evergreen native tree in the New World tropics bearing sweet large berries [MacLeod *et al.*, 1982].

A mixture of long-chain alkyl benzoates with 18, 20, or 22 carbon atoms were present in every

investigated male cuticle extract of *A. compressa*. On the other hand, these compounds were absent in the female cuticle as well as in the DG. The benzoates were identified by comparison of mass spectra and *RI*s between the natural compounds and synthetic references. A blend of alkyl benzoates was prepared in a microreaction by stirring benzoyl chloride and several unbranched long chain alcohols. Also, docosyl benzoate **34** was prepared in 72% yield by coupling benzoic acid and docosanol with *N*-(3-dimethylaminopropyl)-*N*`-ethyl-carbodiimide (EDC) [Patel *et al.*, 1998]. Figure 24 shows the mass spectrum of **34** and Table 3 summarizes the *RI*s for the blend of alkyl benzoates.

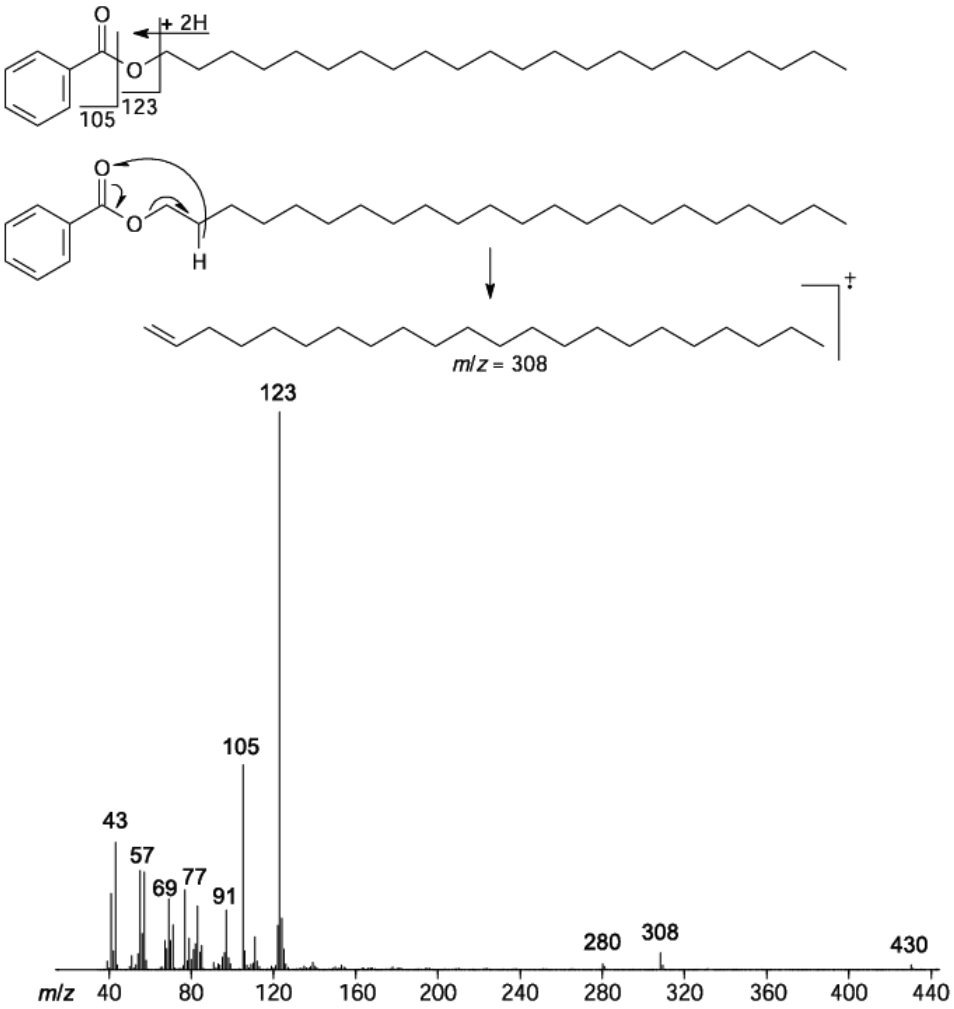

Figure 24: Mass spectrum of docosyl benzoate and important fragmentations.

The ions *m*/*z* = 105 and 123, the latter one forming the base peak, indicated clearly a benzoic acid motif in this compound. The first one was derived from a cleavage of the ester bond and the second one was formed by a McLafferty rearrangement plus hydrogen transfer. An elimination of benzoic acid provided the ion *m*/*z* = 308, reflecting the C22 unit, accompanied by the loss of an ethene fragment to furnish the ion *m*/*z* = 280. The chain length of each alcohol part in these derivatives was deduced from these two fragments. The molecular ion *m*/*z* = 430 was only of low abundance. The

other alkyl benzoates bearing 18 and 20 carbon atoms in the alcohol moiety were also elucidated by the ion pair *m*/*z* = 105, 123 as well as by the ions *m*/*z* = 224, 252 for octadecyl benzoate and 252, 280 for eicosyl benzoate.

The *RI*s of the natural compounds were compared with those of the unbranched alkyl benzoates. The *RI*s of alkyl benzoates depend linearly on the number of carbon atoms in the alcohol part of as shown in Figure 25 and the *RI* of any alkyl benzoate on a BPX-5-phase can be calculated by the regression formula y = 963 + 105*x, where y corresponds to the *RI* and x to the number of carbon atoms in the alcohol moiety. The *RI*s 2852, 3064, and 3275 of the natural compounds were in good agreement with those of the corresponding synthetic samples. Thus, methyl branches in the alcohols were ruled out.

Table 3: Compilation of retention indices for a blend of alkyl benzoates (BPX-5-phase).

Compound	*RI*
Hexyl benzoate	1595
Decyl benzoate	2011
Pentadecyl benzoate	2537
Heptadecyl benzoate	2748
Octadecyl benzoate	2854
Eicosyl benzoate	3064
Docosyl benzoate	3273

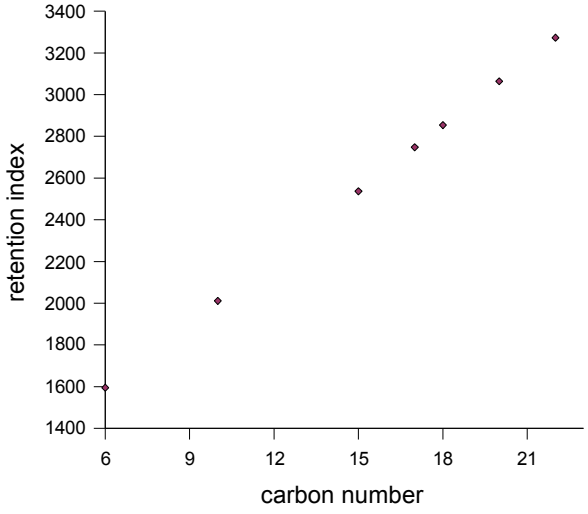

Figure 25: Linear regression analysis for the calculation of the *RI* of alkyl bemzoates on a BPX-5-phase. The *RI* of any derivative is accessible by the formula 963 + 105.0*x.

2.4.6 17-Methylhentriacontan-10-one on the cuticle of *Ampulex compressa* males

Methyl branched long-chain ketones occurred exclusively on the cuticle of male *A. compressa*. The major derivative was 17-methylhentriacontan-10-one, contributing 0.2% to the TIC, accompanied by two minor derivatives also with the keto function at C-10. The positions of the methyl branch in the two minor compounds were not determined because of the low amounts in the natural extracts. The mass spectrum of the major ketone is depicted in Figure 26.

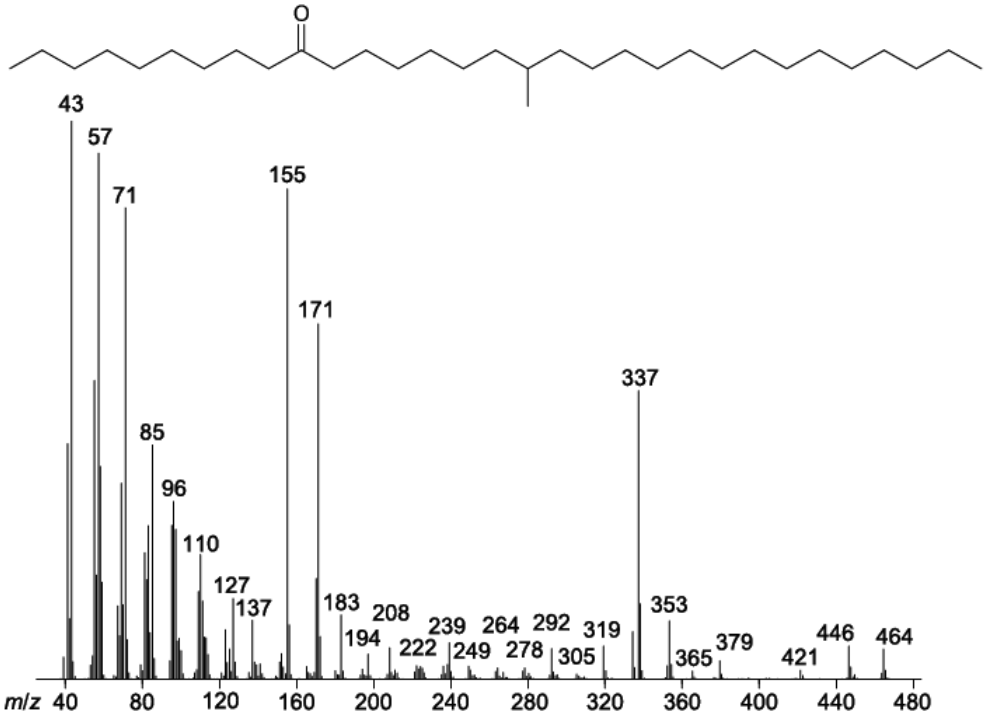

Figure 26: Mass spectrum of 17-methylhentriacontan-10-one.

The mass spectrum was characterized by the molecular ion of 464 amu and a loss of H_2O indicated the presence of an oxygen-containing functional group. Furthermore, the ions *m*/*z* = 155, 171, 337, and 353 were prominent. A ketone seemed to be plausible, because the ions *m*/*z* = 155 and 337 were explained by α-cleavages in both directions. Accordingly, the ions *m*/*z* = 171 and 353 were formed by McLafferty rearrangements with an additional hydrogen transfer. Furthermore, a ketone was also likely because of the occurrence of the ion *m*/*z* = 58, characteristic for ketones. This ion was the final product of two consecutive McLafferty rearrangements. The keto function was located in position 10 if no methyl branches existed in the shorter fragment. Besides this major ketone, two minor ketones were found most likely bearing the keto function in the same position. The mass spectra of the minor derivatives contained also the ions *m*/*z* = 155, 171 as well as the ions *m*/*z* = 309, 325 or *m*/*z* = 323, 339.

These ketones had the *RI*s of 3120, 3225, and 3327. Heptacosan-10-one and hentriacontan-10-one

were prepared in a microreaction to clarify if the natural ketones were branched or unbranched. *RI*s of 2883 and 3285 were obtained. Thus, a keto function in 10-position adds an increment of about 185 points to the *RI* of the longest alkyl chain. Comparison of the *RI*s of the reference compounds and natural compounds proved that the natural ketones were branched and an internal methyl group seemed to be probably because the natural compounds eluted 60 points later, typical for internal methyl groups, than the one carbon shorter synthetic ones [Schulz, 2001] .

Unfortunately, the identification of the position of the methyl branch failed by interpretation of the mass spectrum because indicative fragments were not visible. The ketones were therefore converted into the corresponding alkanes via the corresponding tosylhydrazones, followed by reduction with catecholborane and D_2O to furnish a deuterium labeled alkane as illustrated in Figure 27 [Kabalka and Summers, 1981].

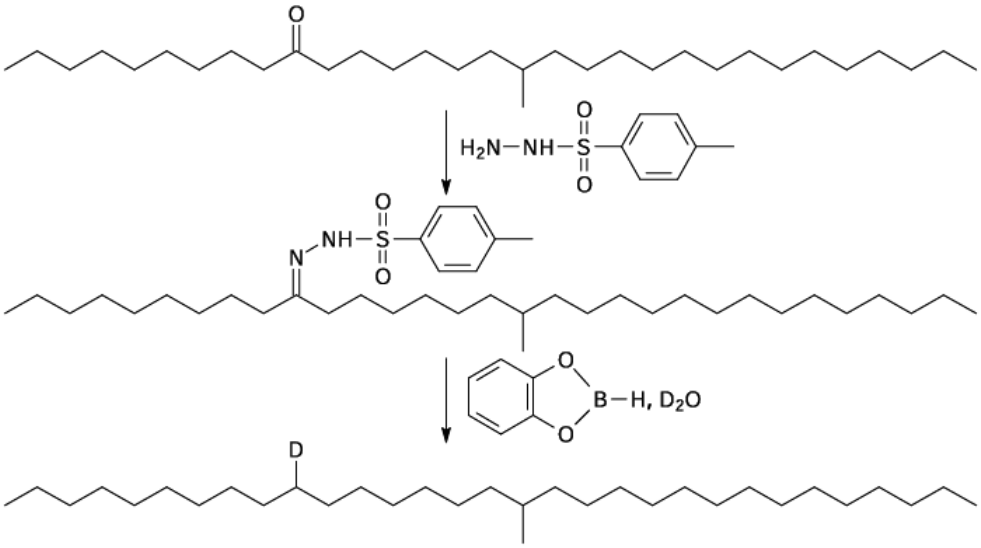

Figure 27: Derivatization scheme for the conversion of 17-methylhentriacontan-10-one into the corresponding labeled 15-methylhentriacontane.

In the case of the major ketone, labeled 15-methylhentriacontane was formed, showing the prominent ions *m*/*z* = 224, 225, 252, and 253. A more detailed analysis of these ions revealed that the ion *m*/*z* = 253 increased in intensity compared to the ion *m*/*z* = 252 indicative for the presence of a deuterium atom in this fragment (Figure 28). Table 4 compares the ratio of intensities between the unlabeled and labeled alkane using spectra at the front and the end of this peak because deuterated compounds elute slightly earlier than the corresponding unlabeled compound.

Figure 28 demonstrates the enhancement of the ion *m*/*z* = 253. The ion *m*/*z* = 253 occurred in 68% intensity relative to the ion *m*/*z* = 252 in the unlabeled alkane whereas this value increased to 88% in the labeled alkane. The ratio of the ions *m*/*z* = 224 and 225 did not alter significantly. This result led

to the conclusion that the keto group, now replaced by the deuterium atom, was initially located in position 10 in the longer fragment relative to the branching point because the mass of the longer fragment was shifted. Hence, 17-methylhentriacontan-10-one was the major ketone. In contrast, an hypothetic shift by 1 amu in the shorter fragment would have been pointed to 15-methylhentriacontan-10-one as structure.

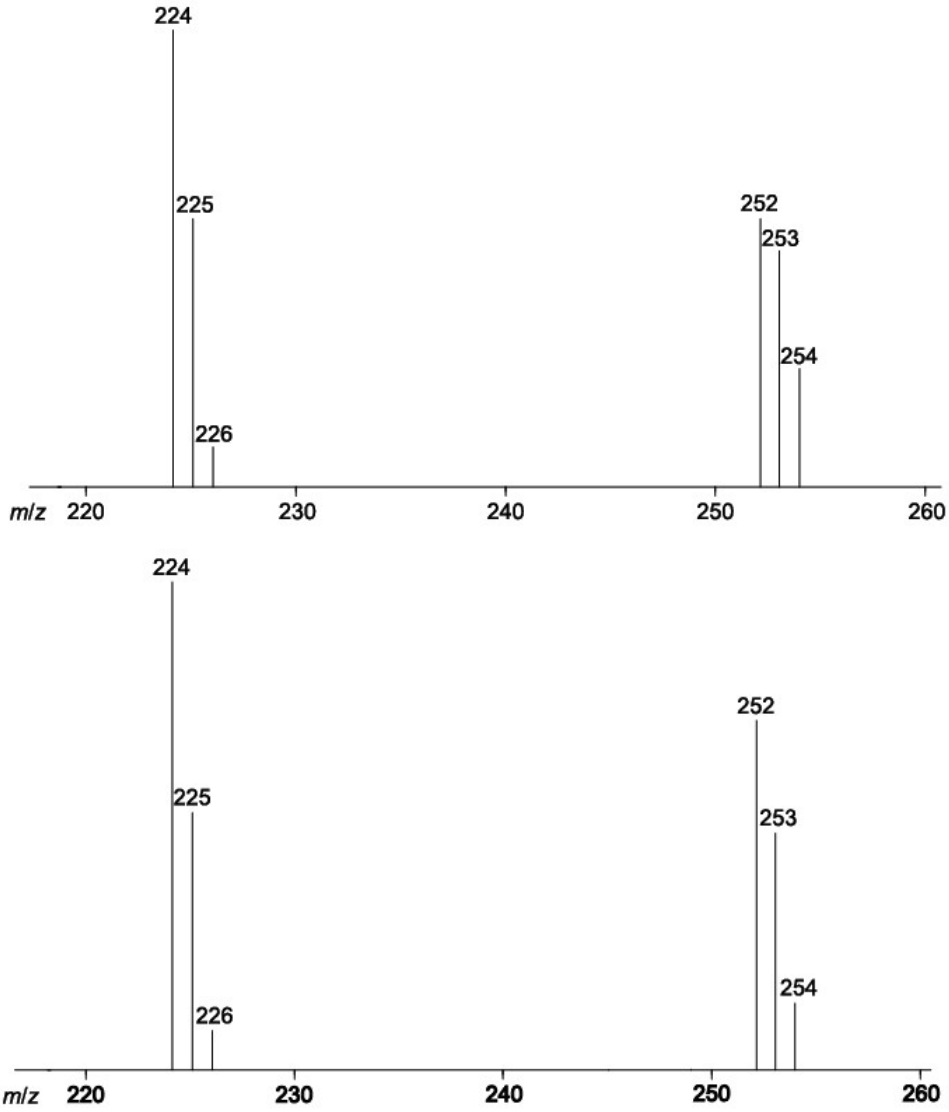

Figure 28: Extracted ion mass spectrum of the ions *m/z* = 224, 225, 226, 252, 253, and 254. Above: labeled 15-methylhentriacontane; below: unlabeled 15-methylhentriacontane.

Table 4: Compilation of the intensities of the ions *m*/*z* = 224, 225, 226, 252, 253, 254, 435, and 436 at the front and back of the peak in the total ion chromatogram.

Front			Back		
m/*z*	Intensity	%	*m*/*z*	Intensity	%
224	3404	100	224	4884	100
225	2001	59	225	2558	52
226	296	9	226	393	8
252	2001	100	252	3473	100
253	1759	88	253	2352	68
254	880	44	254	663	19
435	154	57	435	974	100
436	269	100	436	325	33

2.5 Volatiles released by females and males of the digger wasp *Liris niger*

2.5.1 Previous investigations of *Liris niger*

Table 5 shows the composition of the lipid profile of the DG of female *L. niger* and this profile differed clearly from that of the female cuticle [Gnatzy *et al.*, 2004]. The lipid blend of the cuticle contains long-chain hydrocarbons with 22 to 29 carbon atoms either without or with one and two methyl branches. Alkenes plays a subordinate role. In contrast, the lipids of the DG consist of unsaturated and saturated hydrocarbons with 13 to 18 carbon atoms. Furthermore, saturated and unsaturated acetates are present.

Table 5: Composition of the lipid profile of a Dufour gland extract of *Liris niger* [Gnatzy *et al.*, 2004]. Oform indicates formate as functional group. [a] 8:2 ratio, [b] 1:1 ratio, [c] ratio 5:95 and *E*/*Z* 3:97.

Compound	%
13:H	8.3
14:H	0.4
6ene-, 7ene-15:H[a]	3.5
1ene-15:H	0.3
15:H	55.6
7ene-, 8ene-16:H[b]	Tr
16:H	Tr
6,9diene-17:H	2.9
7ene-, 8ene-17:H [c]	15.6
17:H	1.1

Compound	%
13:OAc	0.5
18:H	Tr
14:OAc	2.5
ene-15:OForm	Tr
15:OForm	0.3
9ene-19:H	Tr
8ene-15:OAc	Tr
19:H	0.5
15:OAc	3.3
7ene-, 9ene-16:OAc	0.5
16:OAc	3.9
8ene-17:OAc	0.4
diene-18:OAc	Tr
9ene-18:OAc	0.5

n-Alkanes account for 66% of a DG extract with 15:H and 13:H as the most abundant components. Additional important compounds are 6,9-heptadecadiene (6,9diene-17:H) and (*Z*)-8-heptadecene (8*Z*-17:H) with 3% and 16%, respectively. The double bond positions were determined by derivatization with DMDS, but the configuration of the double bonds in the 6,9-diene was not clarified. Heptadecene occurs as 95:5 mixture of 8-heptadecene (8ene-17:H) and 7-heptadecene (7ene-17:H) in a *Z/E*-ratio of 97:3. In addition, other internal or terminal are present. Saturated acetates contribute 10% to the TIC whereby the chain length comprise 13 to 16 carbon atoms with hexadecyl acetate (16:OAc, 3.9%) as most abundant representative. Besides several monounsaturated unbranched acetates in quantities lower than 1% were found.

Morphological investigations in *L. niger* led to the assumption that the DG secretion is involved in the chemical communication of thís species. A sexual dimorphism in the morphology of the female and male antennae of *L. niger* was recentyl described [Gnatzy *et al.*, 2006]. The female antennae are composed of 10 flagellomers and the male antennae of 11 flagellomers. In particular, the sensilla trichodea type 3, important for the olfactory perception, is responsible for the sexual dimorphism located ventrally from the 4th to the 9th flagellomer in only a very limited area in females. In contrast, this type of sensilla is exclusively present dorsal from the second to the 11th flagellomer in rich number (over 18.000) in males.

Insect antennae are covered with sensilla exhibiting sense organs. Eight different types of sensilla are described on female and male antennae and they are especially important for mechanical, taste and olfactory perception. The strcuture of a chemical sensilla is drawn in Figure 29 [Leal, 2005].

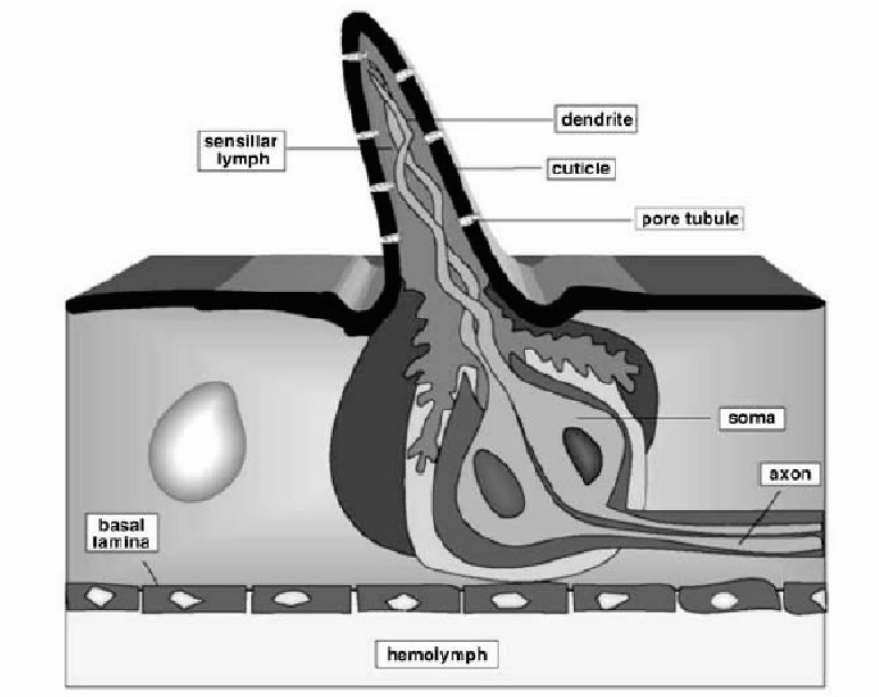

Figure 29: Illustration of a chemical sensilla [Leal, 2005].

The sensilla has a hair-like form. Sense cells in the inner part of the sensilla are proximally connected with an axon and distally with a dendrite. The dendrite forms the inner part of the sensilla and is responsible for the tranlation of the chemical signal into a nervous signal and the transduction of this signal to the sense cells. The sensilla is delimited from the atmosphere by a porous cuticle. Semiochemicals enter the sensilla through these pores and are transported by odorant-binding proteins (OBPs) through the aqueous sensilla lymph to the dendrite. This step is essential because semiochemicals are often lipophilic. The embedding of semiochemicals is an important protection against odorant degrading enzymes. Finally, the semiochemicals are guided to the dendrite by the OBPs. There, the complexes dissociate under release of the semiochemicals by interaction between the OBP and the dendrite membrane, followed by stimulation of the odorant receptors. The odorant receptors convert the chemical signal into a nervous signal and signal transduction proceeds. The axon supports the proximal signal transduction and it is directly associated with the CNS. This signal transduction induces often a visible behavioural trait. The response of an antenna towards chemicals can be recorded in an electroantennogram (EAG) [Leal, 2005]. Therein, the alteration of the electrical conductance of the receptor membrane in the presence of chemicals is measured and these experiments are widespread in pheromone research because they offer the possibility to select active candidates from a set of compounds. In general, the identification of active compounds from a complex mixture is performed by GC-EAG coupled experiments.

2.5.2 Head space analysis of *Liris niger* females and males

HS analyses were performed in OLSA-mode by keeping 4-5 males or females in a glass chamber for 24h in a 12:12-light-dark cycle. Compounds were trapped on a carbon filter, extracted with CH_2Cl_2, followed by GC-MS-analysis. Table 6 compiles the chemical composition of the HS of males, unmated, and mated females of *L. niger*. Figure 30 shows the TICs of the extracts.

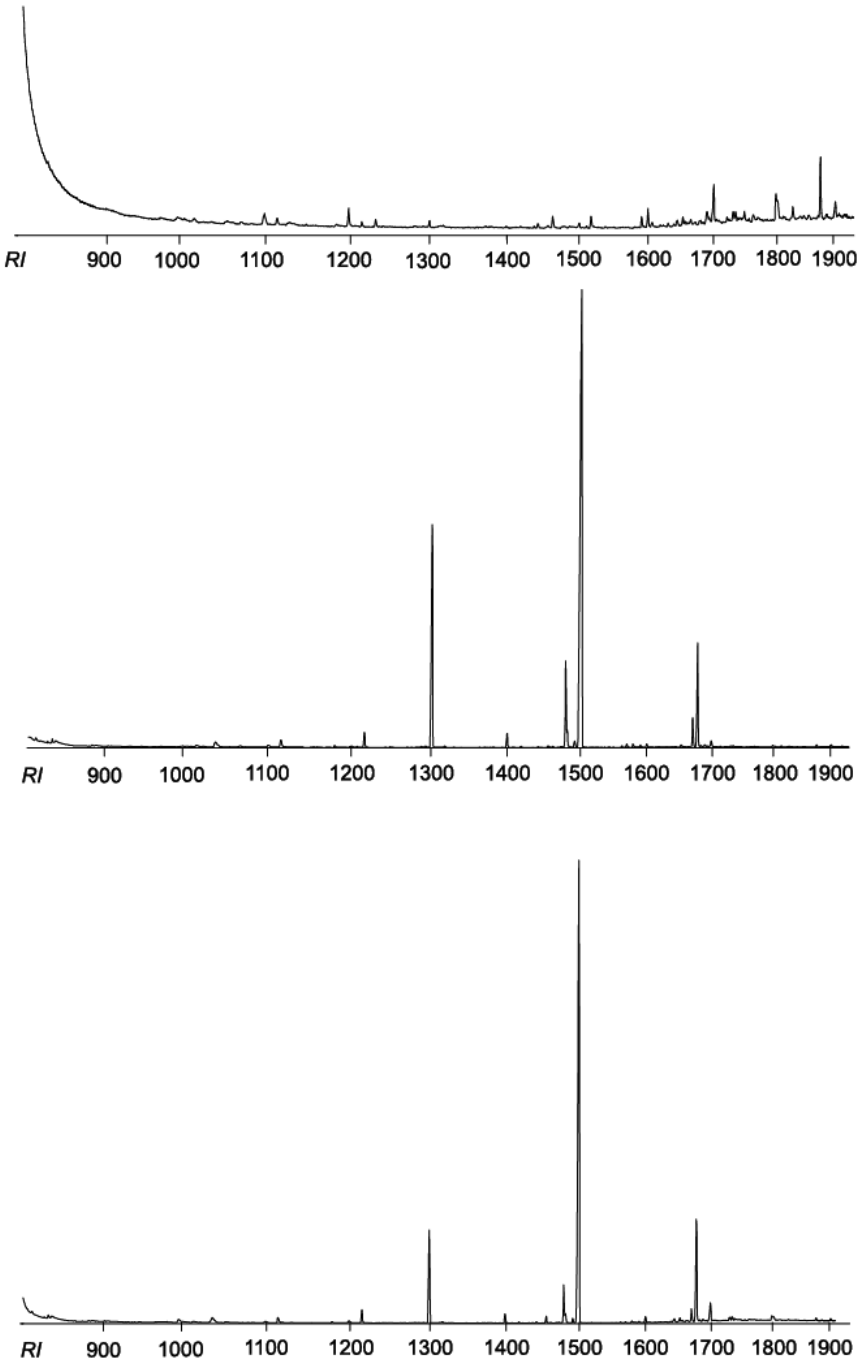

Figure 30: Total ion chromatograms of head space extracts of males, unmated females, and mated females of *L. niger*. Top: males; middle: unmated females; bottom: mated females.

Table 6: Chemical composition of the head space of males, unmated females, and mated females of *L. niger*. Relative intensities of compounds are given in %. uk = unknown

RI	Compound	unmated females (%)	mated females (%)	male (%)
999	Sulcatone	0.2	0.7	-
1016	8:al	0.2	0.4	-
1054	Benzyl alcohol	Tr	Tr	Tr
1068	43, 57, 72, 95, 142; uk	0.2	-	-
1100	11:H	0.3	0.4	Tr
1116	9:al	0.6	0.7	Tr
1131	2-Phenylethanol	-	Tr	-
1180	8Me-9:al	0.2	0.2	-
1198	2-Heptylfurane	Tr	Tr	-
1200	12:H	0.2	0.4	Tr
1208	3,6-DiMe-2-isoButpyrazine	0.1	Tr	-
1216	10:al	1.2	1.4	Tr
1276	2*E*-10:al	Tr	-	-
1287	8Me-10:al	0.1	Tr	-
1294	ene-13:H	„	Tr	-
1300	13:H	16.9	9.2	Tr
1318	11:al	0.1	0.1	-
1364	2Me-13:H	Tr	-	-
1370	3Me-13:H	Tr	-	-
1400	14:H	1.0	0.9	-
1420	12:al	0.1	0.1	-
1433	Ionone	-	Tr	-
1457	Geranylacetone	0.1	0.6	-
1477	6,9diene-15:H	0.1	-	-
1481	6ene- (7ene)-15:H	7.1	4.4	-
1494	1ene-15:H	0.5	0.4	-
1500	15:H	59.0	61.9	Tr
1521	13:al	Tr	Tr	-
1564	2Me-15:H	0.1	Tr	-
1571	3Me-15:H	0.2	0.1	-
1573	6,9-16:H	Tr	-	-
1580	7ene (8ene)-16:H	0.2	0.3	-
1600	16:H	0.2	0.6	-
1622	14:al	-	Tr	-
1644	2,6,10TriMe-15:H	Tr	0.5	Tr
1663	2Me-16:H	-	0.3	-
1671	6*Z*,9*Z*-17:H	2.1	1.3	-
1678	7ene- (8ene)-17:H	7.7	10.5	-
1683	diene-17:H	0.1	0.2	-
1684	ene-17:H	„	„	-
1700	17:H	0.5	2.2	44.0

RI	Compound	unmated females (%)	mated females (%)	male (%)
1714	13:OAc	0.1	-	-
1763	2Me-17:H	-	0.2	-
1771	3Me-17:H	Tr	0.1	-
1800	18:H	0.2	0.5	56.0
1802	2,6,10,14TetraMe-16:H	„	0.5	„
1814	14:OAc	0.1	-	-
1900	19:H	0.1	0.3	Tr

Males released volatiles only in small quantities compared to females and the extract comprised several artifacts like phthalates and siloxanes. Unbranched aldehydes and *n*-alkanes were identified as volatiles in this blend. Heptadecane (17:H) and octadecane (18:H) were the major compounds. Major aldehydes were nonanal (9:al) and decanal (10:al).
The volatile mixture of unmated and mated males consisted of several *n*-aldehydes, (*ω*-1)- and (ω-2)-branched aldehydes, branched and unbranched alkanes, acetates, and a pyrazine.
Unbranched aldehydes occurred with a chain length between 9 and 13 carbon atoms with 9:al and 10:al as most abundant representatives.
The structures of branched aldehydes, merely minor compounds in the extracts of mated females, were deduced by thorough analysis of their mass spectra and their *RI*s. The impact of methyl groups in (*ω*-1)- or (ω-2)-position as well as internal methyl groups on the *RI* of lipoid compounds is well-known [Schulz, 2001]. The structure of 8Me-9:al was confirmed by synthesis followed by comparison of *RI*s and mass spectra between natural and synthetic compound. The mass spectrum of this compound is shown in Figure 31.
The ions *m*/*z* = 128 and 138, formed by the loss of H_2O and ethene, exhibited the expected ions for an aldehyde. The compound eluted 36 points before 10:al pointing to an isomer with a methyl group in (*ω*-1)-position. Therefore, compound **40** was synthesized according to Figure 32 to confirm this proposal. Treating of 1,7-heptanediol **35** in 48% aqueous HBr provided the ω-bromo alcohol **36** in 38% yield, followed by conversion to the corresonding THP-ether **37** in 83% yield [Jayasuria *et al.*, 1990]. Then, **37** was alkylated with isopropylmagnesium chloride in the presence of Li_2CuCl_4 to provide derivative **38** in 66% yield [Tamura and Kochi, 1971]. Deprotection of **38** furnished the (*ω*-1)-branched alcohol **39** in 91% yield. Target compound **40** was obtained by oxidation with PCC in 67% yield [Raederstorff *et al.*, 1987; Easton *et al.*, 2001]. This compound proved to be identical to the natural compound.

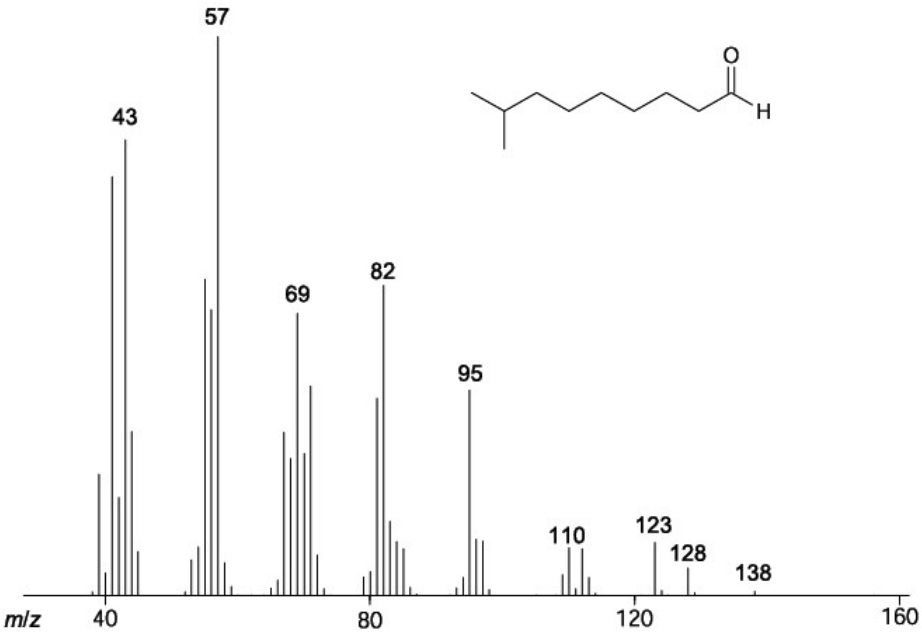

Figure 31: Mass spectrum of 8-methylnonanal.

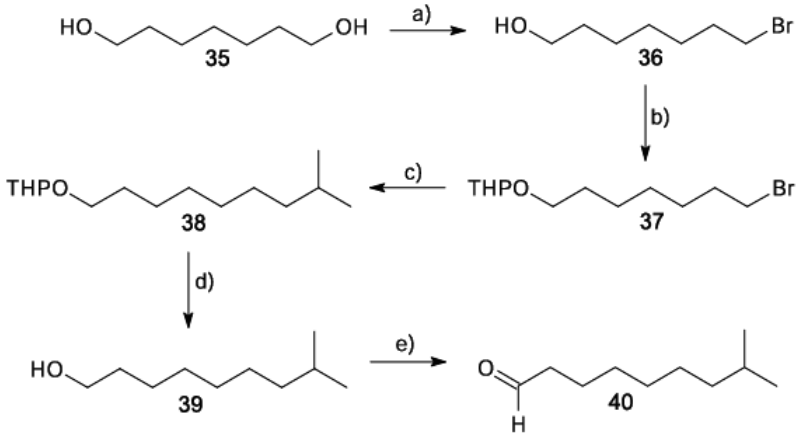

Figure 32: Synthesis of 8-methylnonanal. a) 48% HBr in H_2O, 4h reflux, 38%; b) 3,4-dihydropyrane, *p*-TsOH, Et_2O, rt overnight, 83%; c) *i*PrMgCl, Li_2CuCl_4, 0°C, 24h rt, 66%; d) *p*-TsOH, MeOH, rt overnight, 91%; e) PCC, CH_2Cl_2, 24h rt, 66%.

The structure of 8Me-10:al was elucidated by the ions *m*/*z* = 142 and 152 as well as by a decreased *RI* of 31 points compared to undecanal (11:al).

These aldehydes are known from *Citrus junos*, a plant located in central China with a sour taste. Aldehydes are important components in essential oils of citrus fruits and they are responsible for the odour because of low thresholds [Tajima *et al.*,1990]. In addition, **40** occurs as constituent of the anal gland of the rabbit *Oryctolagus cuniculus*; it was postulated that this and other compounds are involved in territorial defense [Goodrich *et al.*, 1978]. The exocrine secretion of the red hartebeest

is also a source of **40** and similar to the latter example, an implication in territorial defense was assumed [Reiter *et al.*, 2003].

Among these aldehydes, two multiple branched alkanes, 2,6,10-trimethylpentadecane (2,6,10TriMe-15:H) and phytane (2,6,10,14TetraMe-16:H), were present as trace compounds in the HS of unmated females and in higher concentrations (each with 0.5%) in the HS of mated females. These structures were confirmed by comparison with reference mass spectra and also by comparison of their *RI*s with theoretical ones [Nist Database, Schulz, 2001].

These kind of compounds were often reported to be part of the alkane blend of sediments and petroleum accompanied by less branched alkane derivatives and it is assumed that plants and bacteria are forming them [Maxwell *et al.*, 1971]. Furthermore, both compounds are present in the anogenital gland secretion of the giant panda, *Ailuropoda melanoleuca* [Dingzhen *et al.*, 2006].

The volatile blend of unmated females contained a few compounds absent or just present in traces in mated females. These compounds comprised a pyrazine derivative and two straight chain acetates. In addition, (6*Z*,9*Z*)-6,9-heptadecadiene (6*Z*,9*Z*-17:H) occurred in higher concentration in unmated females than in mated ones. Pyrazines are especially important as semiochemicals in ants [Attygalle and Morgan, 1984]. Furthermore, pyrazines seem to be marking volatiles in philanthine and nyssonine wasps [Borg-Karlson and Tengö, 1980]. Straight chain acetates are formed as exocrine constituents in two related species of giant honeybees and furthermore as compounds in the DG of the formicine ant *Lasius niger*, but convincing evidence for their bioactivity is absent [Attygalle *et al.*, 1987; Blum *et al.*, 2000]. The importance of alkadienes as semiochemicals in hymenoptera was decribed earlier in this thesis.

In conclusion, these compounds were of special interest because they occurred preferentially in virgin females and therefore it was investigated if they play a role in the chemical communication of this species.

2.5.3 Compounds preferentially occurring in unmated females and EAG-experiments

2.5.3.1 Structure elucidation of 3,6-dimethyl-2-isobutylpyrazine and EAG-experiments

This pyrazine was characterized by the mass spectrum in Figure 33 and this one was very similar to the mass spectra of several dimethyl-isobutyl pyrazines isomers [NIST Database].

The ion m/z = 122 was the base ion of the mass spectrum and it was formed by a loss of propene from the molecular ion m/z = 164 via a McLafferty rearrangement. The ion m/z = 149 was formed by a loss of a methyl group. Further fragment ions were just of subordinate importance.

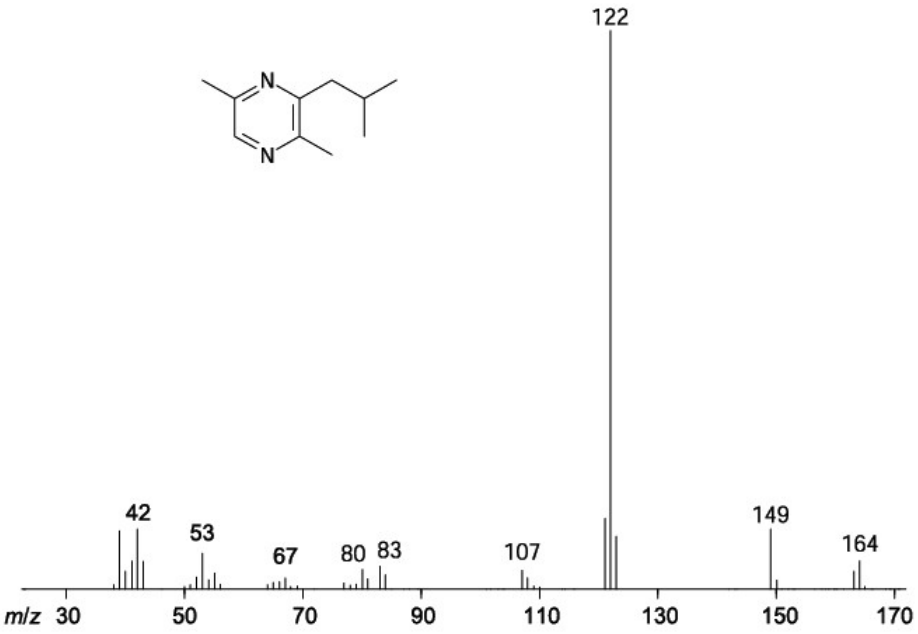

Figure 33: Mass spectrum of 3,6-dimethyl-2-isobutylpyrzine.

The published *RI*s for all three isomers are summarized in Figure 34, determined on a SE-54 capillary column comparable in polarity with the BPX-5 column used in these experiments [Wagner *et al.*, 1999].

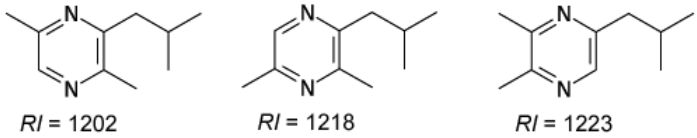

Figure 34: *RI*s of dimethyl-isobutylpyrazine isomers determined on a SE-54 capillary column [Wagner *et al.*, 1999].

The pyrazine derivative of the natural extract was characterized by a *RI* of 1208 and matched that of 3,6-dimethyl-2-isobutylpyrazine with 1202. The *RI*s of the remaining two isomers deviated clearly from the first one. This assignment was verified by comparison the mass spectrum and the *RI* of the natural compound with those of a synthetic sample synthesized by Dickschat [Dickschat *et al.*, 2005a].

This pyrazine was identified as a constituent of the volatile blend of the myxobacterium *Chondromyces crocatus* and marine bacteria [Dickschat *et al.*, 2005a]. This compound is also part of the mandibular gland secretion of ponerine ants from West Africa accompanied by the isomer with the 3,5-arrangement of the methyl groups [Longhurst *et al.*, 1978]. The isomer with the 2,3-arrangement of the methyl groups was described as trail pheromone in the ant

Eutetramorium mocquerysi as part of the secretion of the poison gland [Tentschert *et al.*, 2000].

Pyrazines smells intensively and they exhibit earthy, nutty or roasty odors and many derivatives have thresholds lower than 1 ng/l air [Wagner *et al.*, 1999]. In contrast, 3,6-dimethyl-2-isobutylpyrazine has a threshold higher than 2000 ng/l air similar to the other isomers.

3,6-Dimethyl-2-isobutylpyrazine accounted for 0.1% of the TIC of HS extracts of unmated females, whereas this compound was detected only in traces in analogous extracts of mated females (Figure 35).

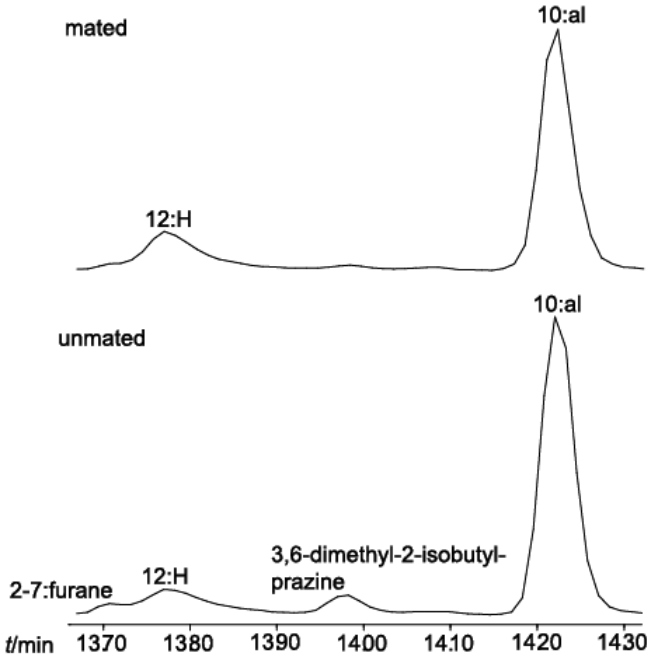

Figure 35: Parts of total ion chromatograms. Top: mated; bottom: unmated females of *Liris niger*.

This compound does not occur as constituent of the DG and hence, another gland must be responsible as releaser of this compound. Pyrazines are often identified in the mandibular gland and the secretion of this gland is particularly involved in defense strategies or in attraction of conspecific sexes [Borg-Karlson and Tengö, 1980; Attygale and Morgan, 1984; Sledge *et al.*, 1999].

Sulphides, ketones, alcohols, aldehydes, terpenes, lactones, and aromatic compounds can comprise additional organic compounds of the mandibular gland [Attygalle and Morgan, 1984]. Hence, an extract of the unmated female head was investigated and therein 3,6-dimethyl-2-isobutylpyrazine was identified as single volatile compound in a complex blend of alkanes. Further experiments are required to elucidate the exact site of the production of this pyrazine.

Based on these observations, this compound as well as an extract of the female head were tested in EAG-experiments with male antennae of *L. niger* by Dr Weissbecker. The experiments were

performed by blowing air over paraffin solutions of the pyrazine and then over the male antennae. Control experiments with paraffin and air induced average responses lower than 0.1 mV. Three different dilutions of the pyrazine in paraffin with concentrations of 0.0015 mol*l^{-1} , 0.015 mol*l^{-1}, and 0.15 mol*l^{-1} were tested and the results are shown in Figure 36. The experiments revealed a dose-dependent response of the male antennae. The less concentrated sample of the pyrazine evoked a voltage of 0.14 mV whereas the more concentrated samples caused a voltage of 0.21 and 0.83 mV, respectively and thus, the threshold value for this compound is at least 0.0015 mol*l^{-1}.

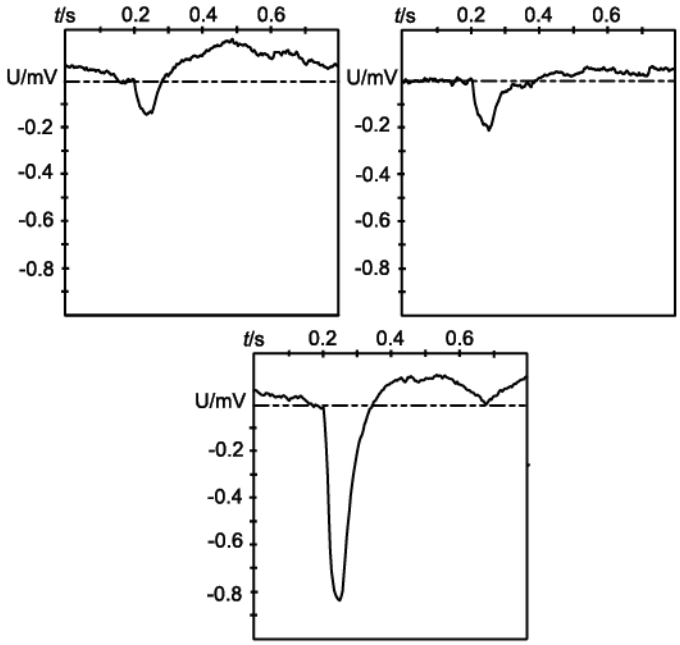

Figure 36: Response of a male antenna to different concentrations of 3,6-dimethyl-2-isobutylpyrazine. Left: 0.0015 mol*l^{-1}; right: 0.015 mol*l^{-1}; bottom: 0.15 mol*l^{-1}.

A head extract also released a response by the male antenna with a voltage of 0.23 mV Figure 37). This extract contained the pyrazine only as trace compound and perhaps a stronger response was prevented by the presence of alkanes.

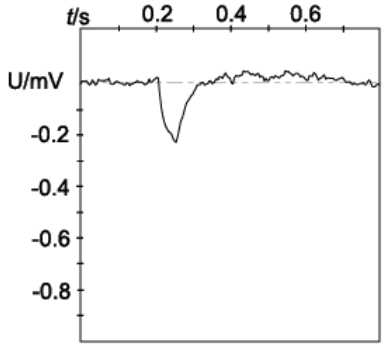

Figure 37: Response of a male antenna to a head extract of *Liris niger* females.

2.5.3.2 Structure elucidation of tridecyl and tetradecyl acetate and EAG-experiments

The mass spectra of the acetates are depicted in the Figures 38 and 39.

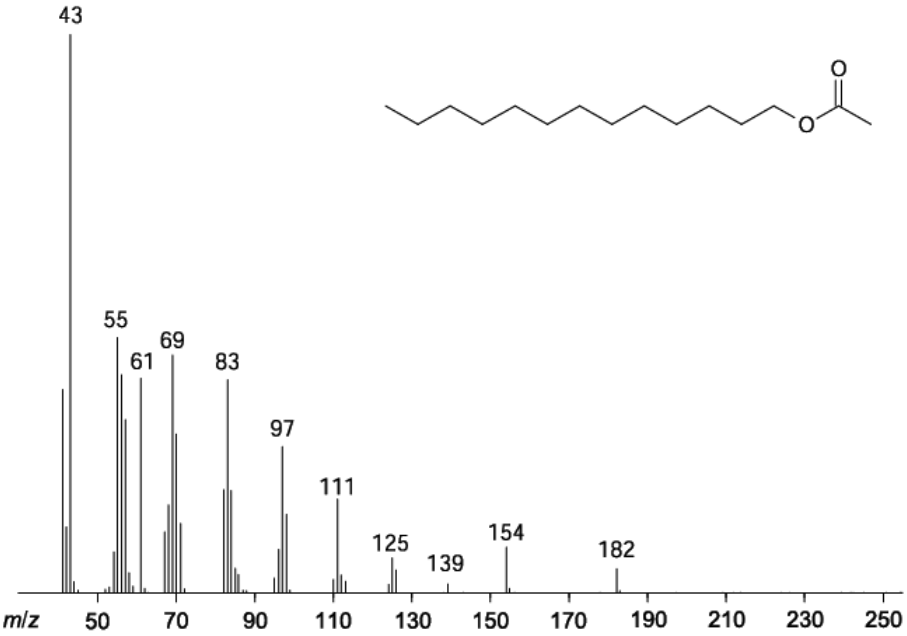

Figure 38: Mass spectrum of tridecyl acetate.

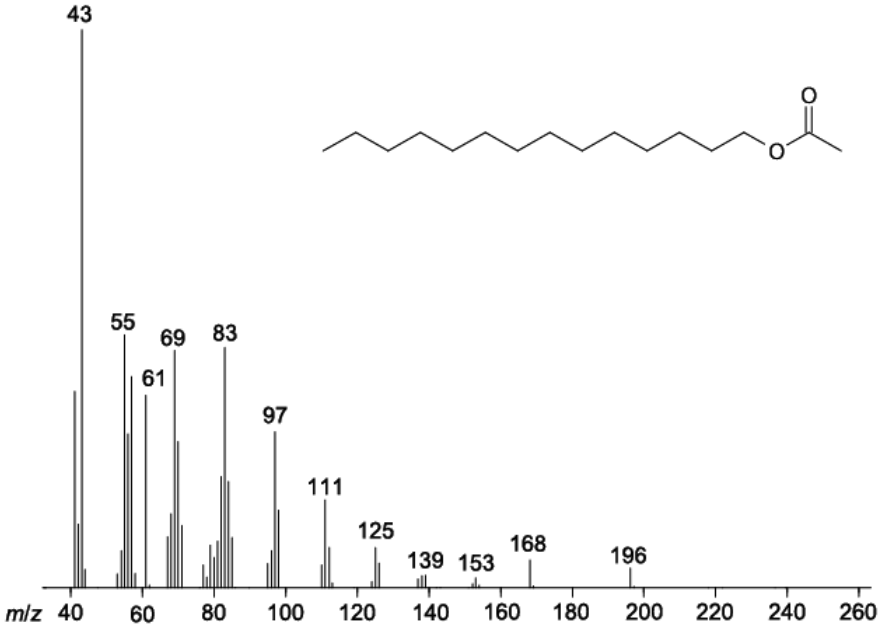

Figure 39: Mass spectrum of tetradecyl acetate.

Both mass spectra contained the ions *m*/*z* = 43 and 61, characteristic for acetates. These are formed by cleavages of the ester bond resulting in the formation of an acylium ion or acetic acid with a additional hydrogen transfer. The molecular ions of both derivatives were only slightly visible, but confirmed their molecular weights of 242 and 256 amu. An elimination of acetic acid followed by the cleavage of ethene provided the ion pairs *m*/*z* = 154, 182 and 168, 196. These fragments allowed

the determination of the chain length of the alcohol moiety. Based on this fragmentation, tridecyl acetate (13:OAc) and tetradecyl acetate (14:OAc) were proposed.

Methyl branches in these compounds were ruled out by the comparison of the *RI*s of synthetic samples (1710 and 1808) and the natural compounds (1714 and 1814). Synthetic samples deviated only slightly with values of 1710 and 1808. Synthetic samples of tridecyl acetate **41** and tetradecyl acetate **42** were synthesized by esterification of acetyl chloride in 89% yield. Both derivatives were identified as constituents of a complex mixture of lipid derivatives in the DG of ants before [Bagnères *et al.*, 1991, Gökçen *et al.*, 2002].

Synthetic samples of these acetates, applied in concentrations of 0.09 mol*l^{-1}, elicited responses of the male antenna in the range of the background responses of air and paraffin with 0.08 mV and thus, they do not trigger an electrophysiological response.

2.5.4 EAG-experiments with (9*Z*,12*Z*)-9,12-octadecadienyl acetate

Compound **43**, (9*Z*,12*Z*)-9,12-octadecadienyl acetate was also identified as content of the DG. This compound was synthesized according to Figure 40.

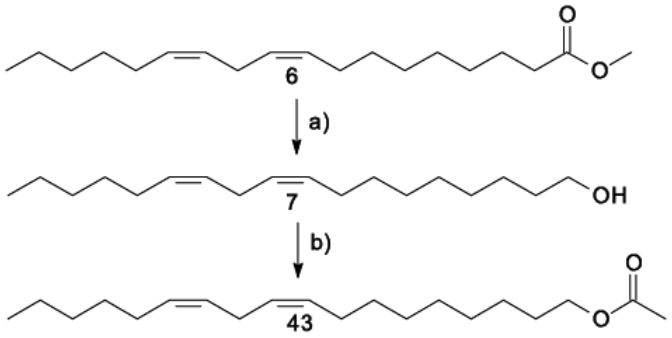

Figure 40: Synthesis of (9*Z*,12*Z*)-9,12-octadecadienyl acetate. a) $LiAlH_4$, Et_2O, overnight rt, 80%; b) AcCl, pyridine, 3h rt, 93%.

First, methyl linoleate **6** was reduced in the presence of $LiAlH_4$ to furnish alcohol **7** in 80% yield. Then, **7** was converted to the acetate **43** in 93% yield by esterification with acetyl chloride.

This compound evoked a weak response of 0.07 mV which was similar to the responses of the other acetates and the range of the background noise.

2.5.5 EAG-experiments with saturated and unsaturated hydrocarbons of the Dufour gland

Tridecane (13:H) and tetradecane (14:H) contributed 16.9% and 1.0% to the TIC of the HS extract. The amount of 13:H decreased to 9.2% after mating whereas the amount of 14:H remained nearly equal with 0.8%. A response of the male antennae was exclusively induced by 13:H and therein a

response by the male antenna of 0.19 mV was recorded (Figure 41). In the case of 14:H, the experiments were less clear and the responses of the male antenna were different in several trials.

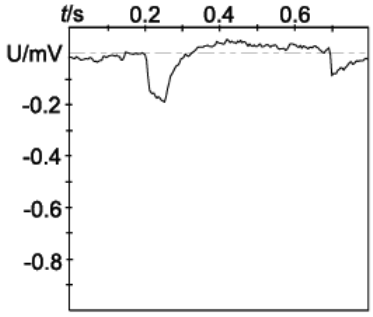

Figure 41: Response of male antenna to tridecane.

Compound **47**, (*Z*)-8-heptadecene (8*Z*-17:H) was synthesized according to Figure 42. Alkylation of non-1-yne **44** with 1-bromooctane **45** furnished compound **46** in 14% isolated yield [Buck and Chong, 2001]. This low yield was caused by problems in the separation of the product from the educts. Final hydrogenation with palladium on carbon provided the target compound **47** in 99% yield.

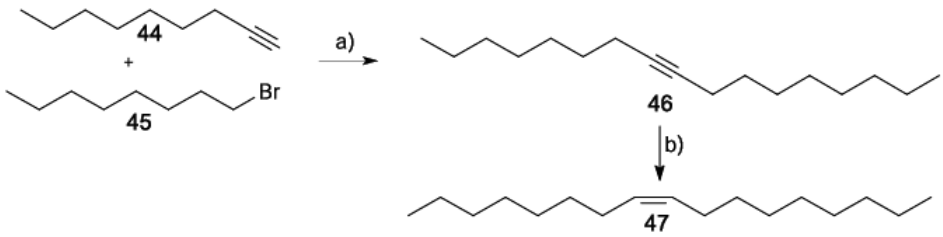

Figure 42: Synthesis of (*Z*)-8-heptadecene. a) -78°C *n*-BuLi, → rt, NaI and 1-bromooctane, reflux 40h, 14%; b) H_2, Pd/C, MeOH, 1bar, 95 min, 99%.

This compound had a content of 7.7 % in unmated females. In contrast, its amount increased in mated ones to 10.5%. The reaction of the male antenna to **47** in a concentration of 0.08 mol*l^{-1} in EAG-experiments was ambiguously because some samples were active whereas other evoked no response. In average, the value of the response was with 0.13 mV only slight greater than the background noise and therefore activity was difficult to elucidate.

In contrast, 1-ene-15:H elicited a response of 0.22 mV in a concentration of 0.12 mol*l^{-1} as illustrated in figure 43. This compound occurred in unmated and mated females in nearly equal quantities.

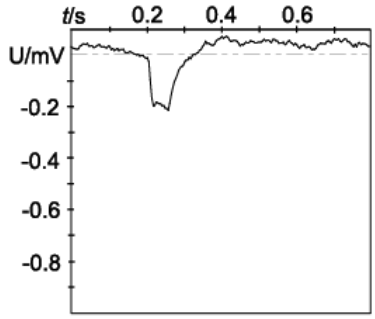

Figure 43: Response of male antenna to 1-pentadecene.

In addition, (6*Z*,9*Z*)-6,9-heptadecadiene (6*Z*,9*Z*-17:H) **52** was tested, being more pronounced in unmated females (2.1%) than in mated ones (1.3%). This compound was prepared by two strategies according to Figures 44 and 45.

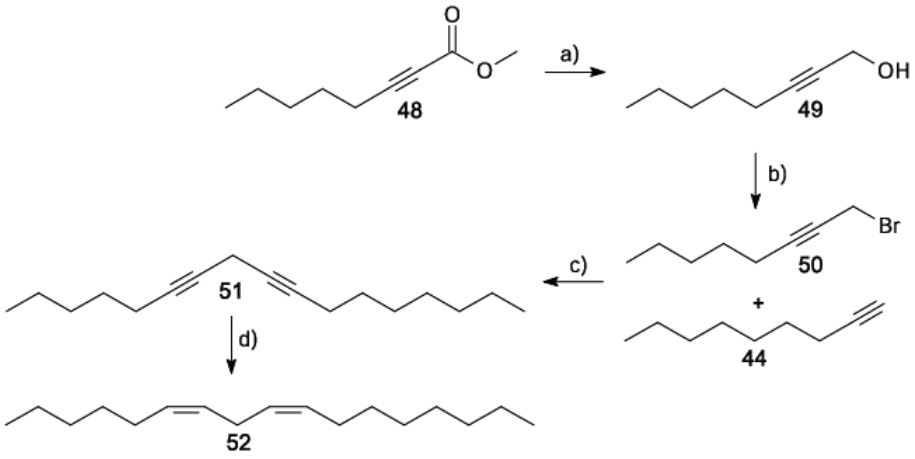

Figure 44: Synthesis of (6*Z*,9*Z*)-6,9-heptadecadiene by an acetylenic approach. a) DIBAL, Et_2O, 3h -78°C, → rt, 93%; b) pyrdine, MsCl, LiBr 1h rt, 35%; c) C_2H_5MgBr 1.5h 45°C, Cu(I) -10°C 20 min, rt overnight, 95%; d) $Ti(OiPr)_4$, *i*PrMgBr, Et_2O, -78°C, → 2h -30°C, → -78°C, H_2O, 5%.

Methyl oct-2-ynoate **48** was reduced at -78°C to the corresponding alcohol **49** by diisobutylaluminium hydride (DIBAL) in 93% yield. Then, **49** was converted to bromide **50** in 35% yield by stirring with pyridine, methane sulfonyl chloride (MsCl) and lithium bromide [Nilsson *et al.*, 1996]. An alternative transformation with quantitative conversion was performed by treating with carbon tetrabromide, but product purification was impaired by coelution of CBr_4 [Gueugnot *et al.*, 1996]. Subsequently, the diyne skeleton **51** was synthesized in 95 % yield in a coupling reaction between **50** and non-1-yne **44** in the presence of Cu(I) [Millar and Underhill, 1986]. Finally, **51** was reduced in the presence of isopropylmagesium bromide, titanium tetraisopropylate at temperatures ranging between -30 and -78°C but **52** was only

obtained in 5% yield [Harada *et al.*, 1995]. GC-analysis revealed that **52** was completely consumed but a not further characterized polymeric compound was formed as main product.

Alternatively, **52** was prepared by a Wittig approach [Pohnert and Boland, 2000]. Therefore, (*Z*)-3-nonenol **53** was converted into the iodide **54** by stirring with iodine, imidazole, and triphenylphosphane in 85% yield. After that, the corresponding Wittig salt **55** was synthesized in 45% yield by heating **54** to reflux with triphenylphosphane in acetonitrile. Final Wittig-reaction with sodium hexamethyldisilylamide (NaHMDS) in dimethoxyethane (DME) at -78°C provided **52** in 81% yield in a *Z*,*Z*:*Z*,*E*-ratio of 94:6 (GC) whereas the acetylenic approach furnished only the (*Z*,*Z*)- isomer.

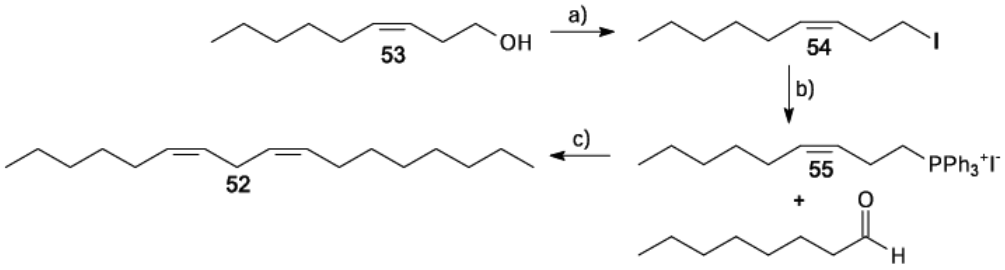

Figure 45: Synthesis of (6*Z*,9*Z*)-6,9-heptadecadiene by a Wittig approach. a) PPh_3, imidazole, I_2, Et_2O:MeCN 3:1, 0°C → rt overnight, 85%; b) PPh_3 , MeCN, 4h reflux, 45%; c) DME, NaHMDS, -78°C, → rt overnight, 81%.

The male antenna responded (0.16 mV) to **52** in a concentration of 0.08 mol*l^{-1} (Figure 46) which means that this compound can be noticed by the male wasp.

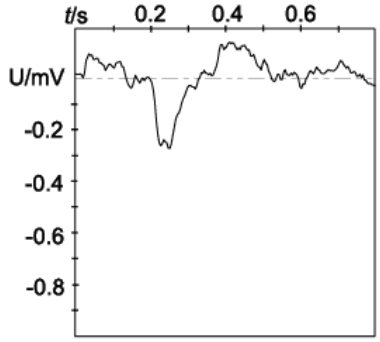

Figure 46: Response of male antenna to (6*Z*,9*Z*)-6,9-heptadecadiene.

2.5.6 EAG-experiments with extracts of the Dufour gland

Several DG preparations were used to investigate their influence on the response on the male antenna and the results of three different EAG-experiments are depcited in Figure 47. The male antenna was sensitive to a DG preparation in water underscored by a distinct reaction with a voltage of 0.65 mV. Possibly, water soluble compounds of the DG were responsible for the reaction of the

male antenna. This response was similar with 0.71 mV after treating the male antenna with the same DG preparation 30 min later. In contrast, unpolar compounds of the DG were also able to evoke a reaction of the male antenna and this fact was underlined by a response of 0.25 mV to a DG extract in CH_2Cl_2. However, this reaction was only weak compared to the aqueous DG preparation. These investigations show that further experiments are needed to identify additional active compounds of the DG. Especially, an analysis of the polar constituents of the DG should be valuable.

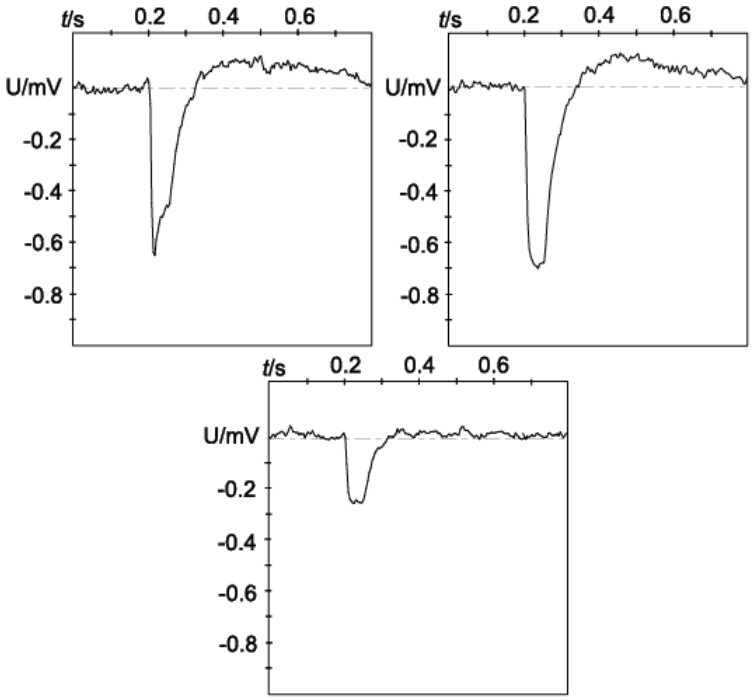

Figure 47: Responses of male antenna to preparations of the Dufour gland. left: DG + H_2O, right: same DG after 30min; bottom: extract of DG in CH_2Cl_2.

3. Identification of novel spider lipids from *Argyrodes elevatus* and *Diplocephalus permixtus*

3.1 New cuticular lipids of *Argyrodes elevatus*

3.1.1 Lifestyle of spiders of the genus *Argyrodes*

Species of the genus *Argyrodes* are mainly kleptoparasites, belong to the familiy theridiidae, and are widespread on earth. Most species are unable to construct webs suitable for prey capture and therefore they rely on a host web for the uptake of nutrition [Bürgis, 1984]. The hosts are variable and either the parasite lives directly in the web of the host or builds a web associated with the host web.

In the case of the interaction with the host spider *Cyrtophora citricola*, *A. argyrodes* lives in the host web. Normally, the intruder is not detected by the host because the parasite is capable to move very carefully in the web during the raids. If prey is caught, the parasite moves to the prey and steel it before the host arrives.

In contrast, in the host web of *Argiope lobata*, *A. argyrodes* weaves a web at the periphery of the host web. Threads of this web are linked with the host web for perception of prey catches. If prey is caught, the host spider starts to feed on the prey. Synchronously, the parasite enters the host web and sneaks up to the prey item opposite to the host spider. Normally, the parasite is not recognized and the host and the parasite consume the prey together.

Smaller orb-webs are a greater challenge for the parasite because its detection by the host is facilitated. Thus, the parasite must develop a more sophisticated strategy for the predation. The parasite cuts through the thread to the host web and bridges the gap with its body followed by coiling up the host web and simultaneously elongating the own thread. This process proceeds up to the detection of the prey and then the parasite snaps the item with its legs and falls off the host web. Alternatively, food bundles are stolen from the pantry of the host.

This interaction between intruder and host does not inevitably discriminate the host because the intruder removes often prey items which are disregarded by the host [Cangialosi, 1990]. In these cases, the interaction is commensal and the intruder acquires nutrition. In return, the host web is cleaned. On the other hand, also detrimental impacts for the host are known by the presence of the intruder. An impaired development of *N. plumipes* was reported by the presence of *A. antipodianus* and 55% less weight gain as well as 4.5 times higher web relocation rates were observed for the host compared to individuals without intruder [Grostal and Walter, 1997]. The intruder can also hunt down the host spider as described in *A. fissifrons* [Tanaka, 1984]. Although the intruder is much smaller than its host *Agelena limbata*, the intruder preys occasionally

upon the host, preferentially during or after moulting of the host.

3.1.2 Composition of the male and female cuticle of *Argyrodes elevatus*

Extracts of the cuticle of male and female heads were investigated by GC-MS and TICs are illustrated in Figure 48.

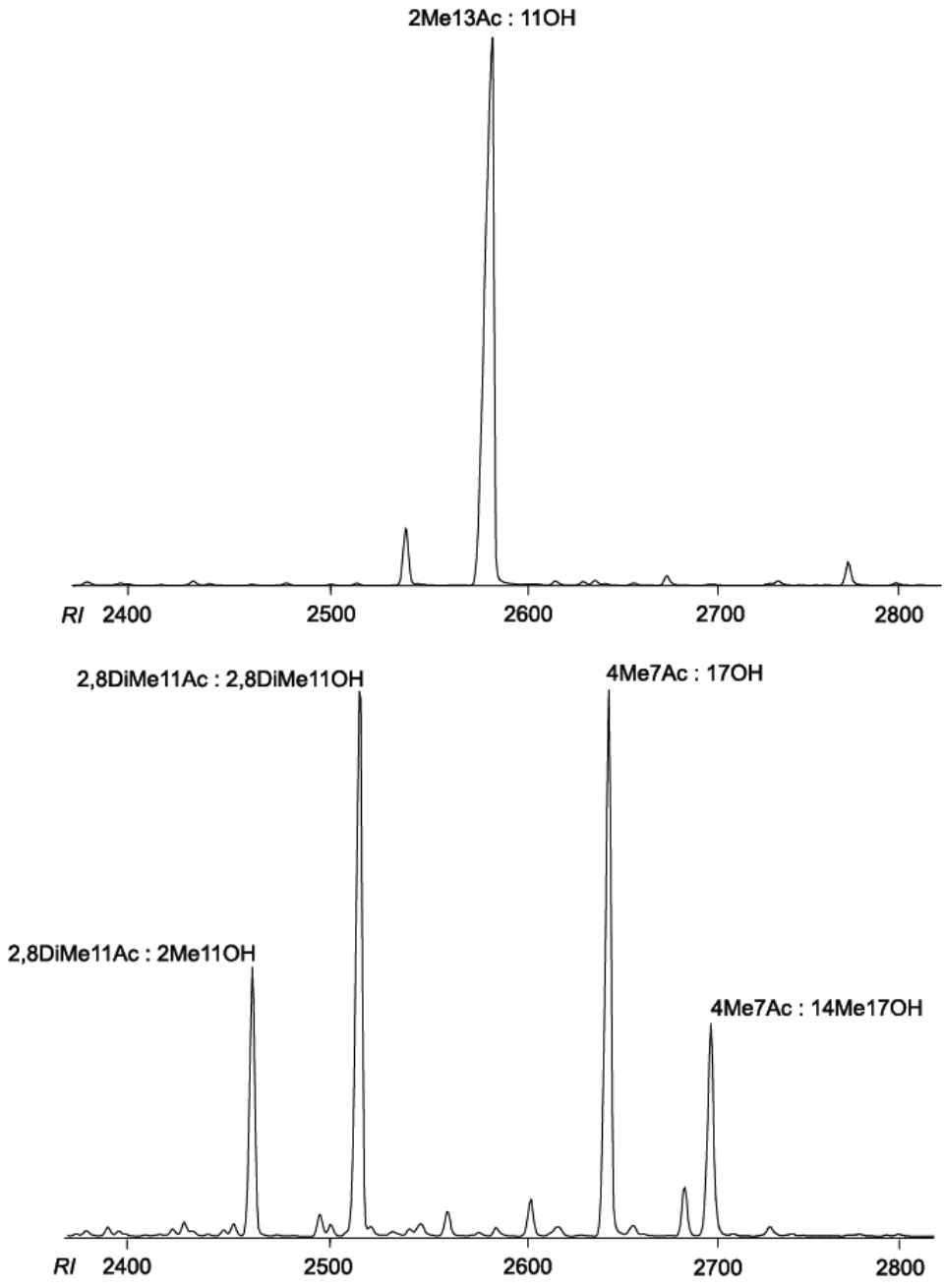

Figure 48: Total ion chromatograms of male and female head extracts of *Argyrodes elevatus*. Top: Male head; Bottom: Female head.

This figure shows clearly that a sexual dimorphism in the cuticular lipid blend exists. Interestingly, the male and female extract contained only a few number of distinct compounds. These compounds were wax esters with methyl branches in the acid parts and occasionally also in the alcohol parts.

The male extract consisted mainly of one wax ester contributing 76.0% to the TIC. This derivative was identified as undecyl 2-methyltridecanoate (2Me13Ac:11OH). Further derivatives of this compound class occurred as trace compounds in this extract. Hydrocarbons, typical members of lipid blends of arthropods, played just a subordinate role.

The corresponding female extract contained preferentially 2,8DiMe11Ac:2Me11OH (11.3%), 2,8DiMe11Ac:2,8DiMe11OH (28.0%), 4Me7Ac:17OH (26.2%), and 4Me7Ac:14Me17OH (10.3%). Other wax esters existed in traces and also hydrocarbons were just of minor importance.

Branched wax esters are characterized by complex mass spectra (70eV) with various fragmentation pathways causing problems in the structure elucidation of these compounds. The mass spectra of the major derivatives are shown in the Figures 51-55.

Besides the molecular ion, the mass spectra of wax esters exhibit three major fragmentation pathways and these will be discussed for the major derivatives in both extracts.

First, an ion pair with the general formulas $C_nH_{2n-1}O^+$ and $C_nH_{2n+1}O_2^+$ is formed. These ions correspond to the acid part of the wax ester and are typical for esters [Budzikiewicz, Djerassi, and Williams, 1967]. α-Cleavage between the carbonyl moiety and the ester oxygen atom furnishes the ion $C_nH_{2n-1}O^+$, while the ion $C_nH_{2n+1}O_2^+$ results from a cleavage between the ester oxygen and the alkyl group with subsequent transfer of two hydrogen atoms to the acid fragment. Second, the molecular formula of the alcohol part of the wax ester is derived by the ion pair with the general formulas $C_nH_{2n}^+$ and $(C_nH_{2n}\text{-}28)^+$ [Budzikiewicz, Djerassi, and Williams, 1967]. These ions are formed by elimination of the acid via McLafferty rearrangement, followed by extrusion of ethene from the alkyl fragment according to Figure 49.

Third, the characteristic ion $m/z = 74$ in the mass spectra of wax esters points to an acid containing a methyl group at C-2. This ion results from a twofold McLafferty rearrangement and this pathway is depicted in Figure 50, also for 2Me13Ac:11OH. Furthermore, the ions $m/z = 87$ and 143 are indicative for acids with a methyl group at C-2 [Budzikiewicz, Djerassi, and Williams, 1967].

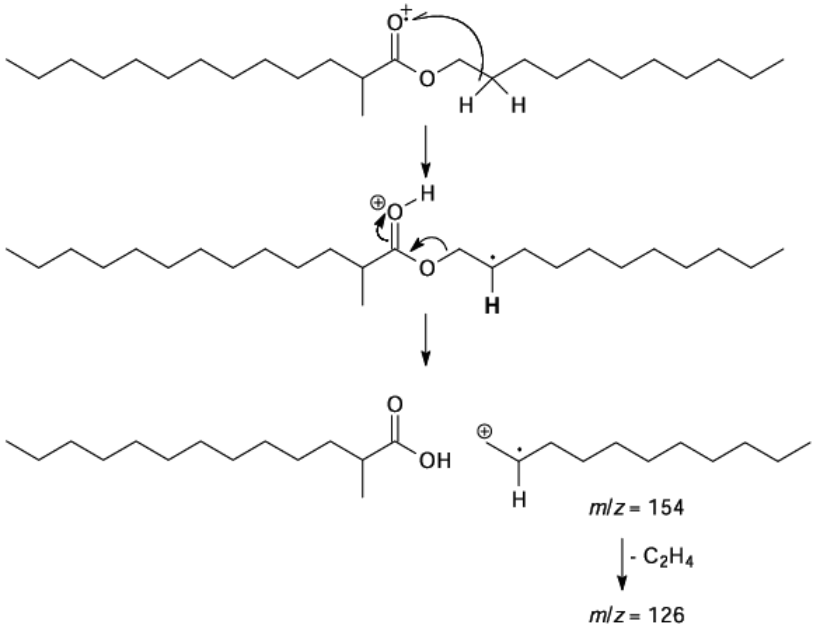

Figure 49: Fragmentation pathway leading to the ions *m*/*z* = 126 and 154 in the mass spectrum of undecyl 2-methyltridecanoate.

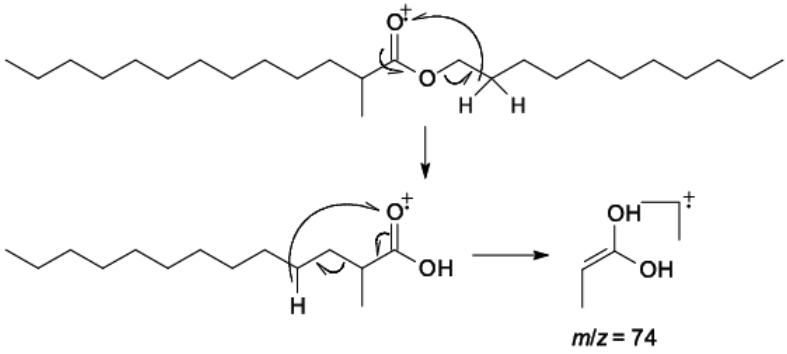

Figure 50: Formation of the ion *m*/*z* = 74 in the mass spectrum of undecyl 2-methyltridecanoate.

The mass spectrum of 2Me13Ac:11OH (Figure 51) was characterized by the ion pair *m*/*z* = 211 and 229. These ions indicated an acid with 14 carbon atoms. The formation of the ions *m*/*z* = 126 and 154 was explained in Figure 49 and these ions pointed to an alcohol part with 11 carbon atoms. Furthermore, the ions *m*/*z* = 74, 87, and 143 indicated a methyl group at C-2 in the acid. The *RI* of this compound was determined to 2581 points.

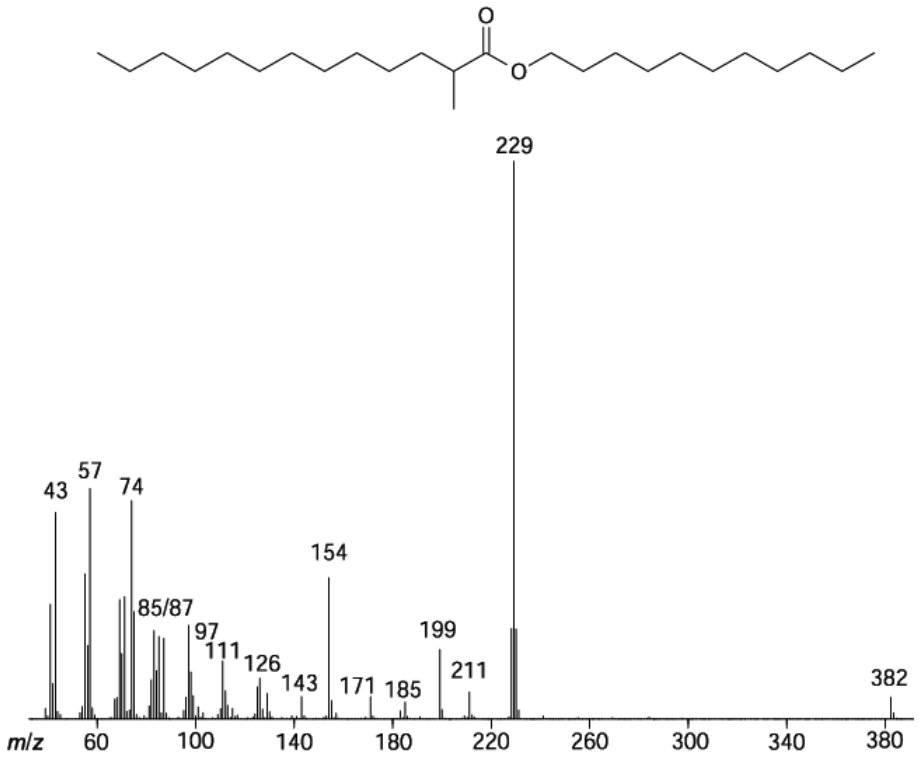

Figure 51: Mass spectrum of undecyl 2-methyltridecanoate.

The mass spectrum of the first eluting wax ester of the female cuticle contained the ions *m*/*z* = 74, 140, 168, 197, as well as 215 and this compound had a *RI* of 2461. The first ion indicated the presence of a 2-methyl acid part and the others that the wax ester consisted of an acid with 13 carbon atoms and an alcohol with 12 carbon atoms (Figure 52).

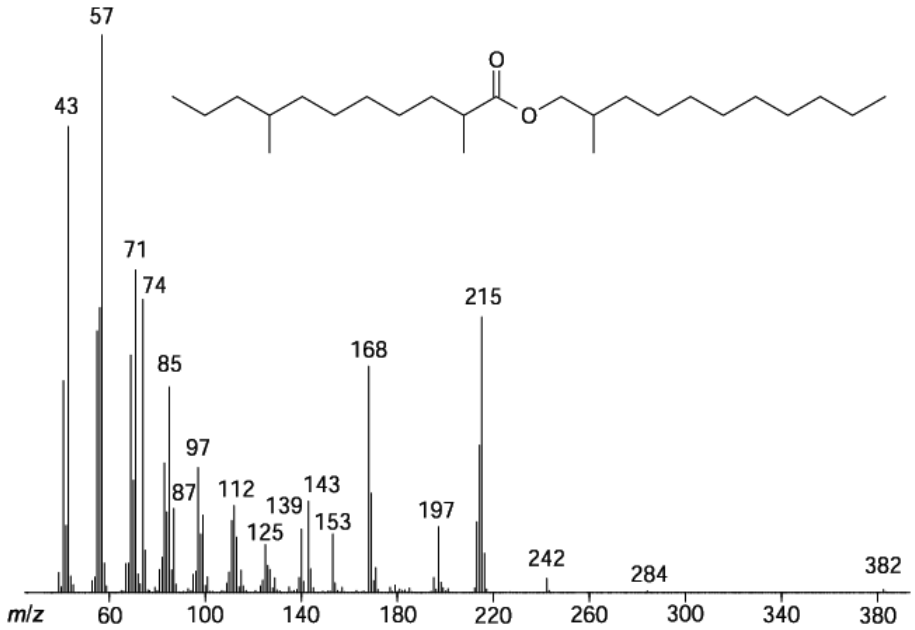

Figure 52: Mass spectrum of 2-methylundecyl 2,8-dimethylundecanoate.

The mass spectrum of the second major compound exhibited the ions *m*/*z* = 74, 154, 182, 197, and 215 indicating a 2-methyl acid with 13 carbon atoms like before but the alcohol consisted of 13 carbon atoms (Figure 53). The *RI* of this wax ester was 2514.

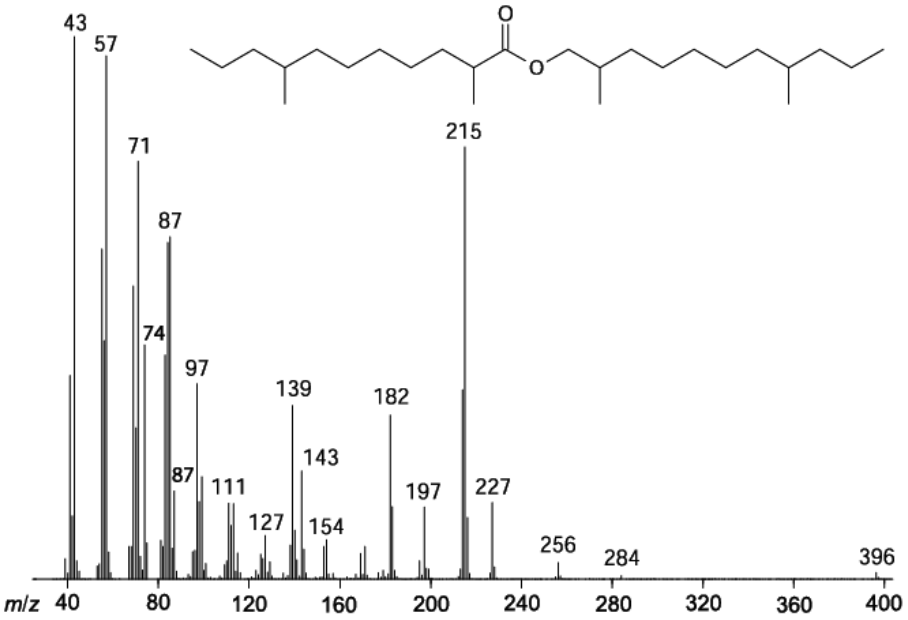

Figure 53: Mass spectrum of 2,8-dimethylundecyl 2,8-dimethylundecanoate .

In contrast, the ion *m*/*z* = 74 was absent in the mass spectra of the last two major derivatives and hence the acids of these wax esters were unbranched at C-2. The third derivative was characterized by the ions *m*/*z* = 127, 145, 210, and 238, and a *RI* of 2642 (Figure 54).

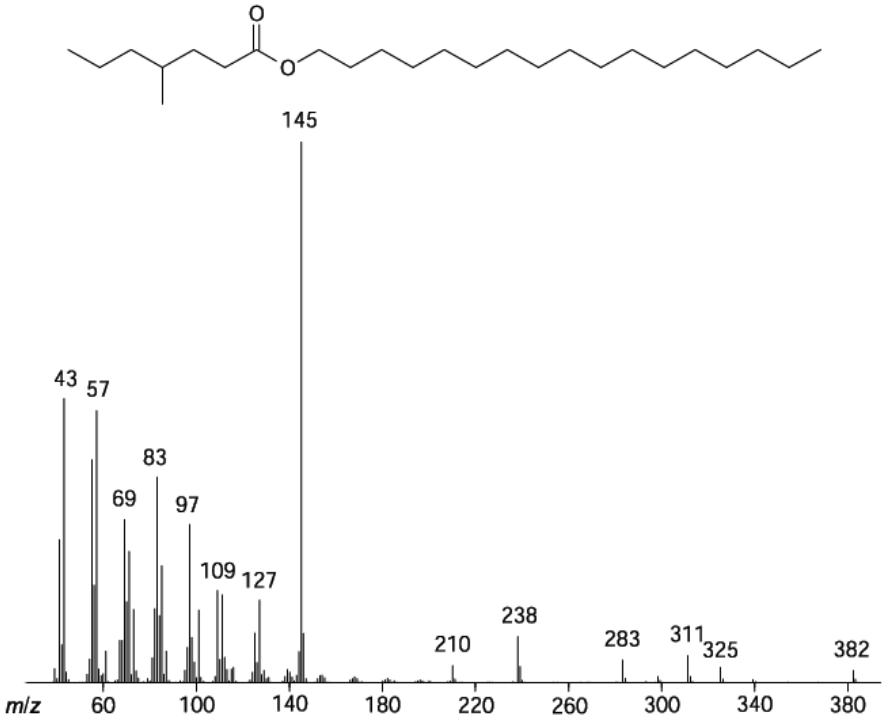

Figure 54: Mass spectrum of heptadecyl 4-methylheptanoate.

This wax ester comprised an acid with 8 carbon atoms and an alcohol with 17 carbon atoms.

The mass spectrum of the fourth major female wax ester also contained an acid with 8 carbon atoms, indicated by the ions *m*/*z* = 127 and 145 whereas the alcohol had 18 carbon atoms (Figure 55). This compound had a *RI* of 2697.

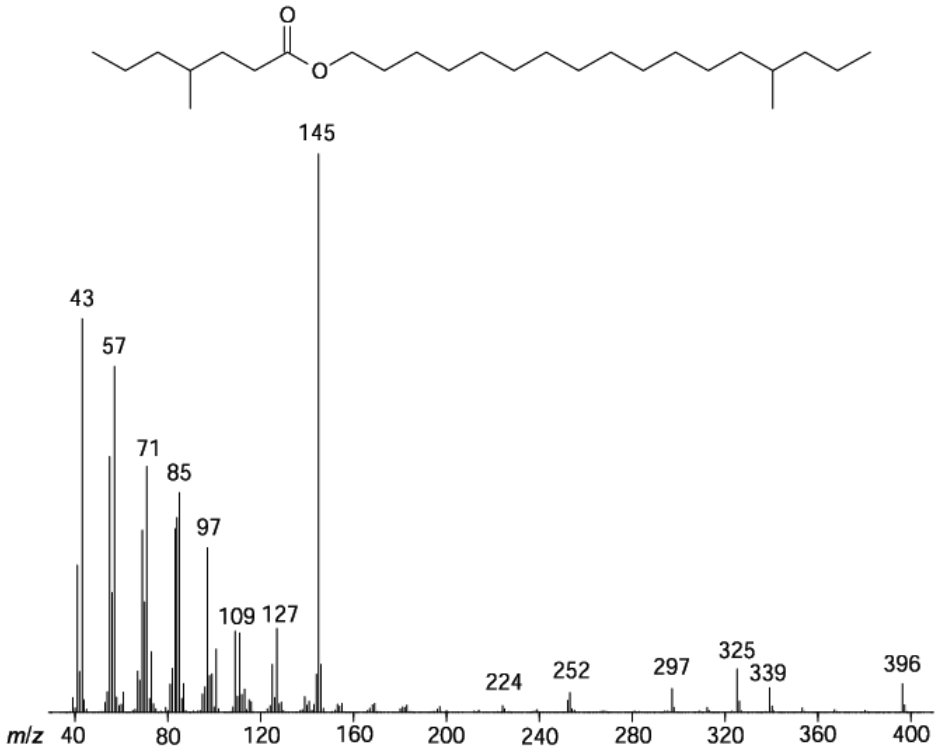

Figure 55: Mass spectrum of 14-methylheptadecyl 4-methylheptanoate.

3.1.3 Structure elucidation of the wax esters by derivatizations

3.1.3.1 Flow scheme for the analysis of wax esters

The structure elucidation was performed as shown in Figure 56.

Wax esters were transesterified with trimethylsulfonium hydroxide (TMSH) under formation of methyl esters and the free alcohols. The *RI* of methyl branched methyl esters on a BPX-5-phase can be calculated by the formula $RI = I_{\text{Chain length Acid}} + I_{\text{Methyl ester}} + I_{\text{position, Methyl group}}$ under utilization of the increment data in Table 7 [Khorasheh *et al.*, 1989; Schulz, 2001]. That means that the *RI* of a branched methyl ester on a BPX-5-phase can be assessed by summing up the increments for the longest alkyl chain in the methyl ester, the methyl ester group, and the position of the methyl group in the alkyl chain. In general, the increment for the methyl branch increases towards the ω-end and values are compiled in table 7. The increment for the methyl ester group was characterized by a *RI* of 323 during these analyses.

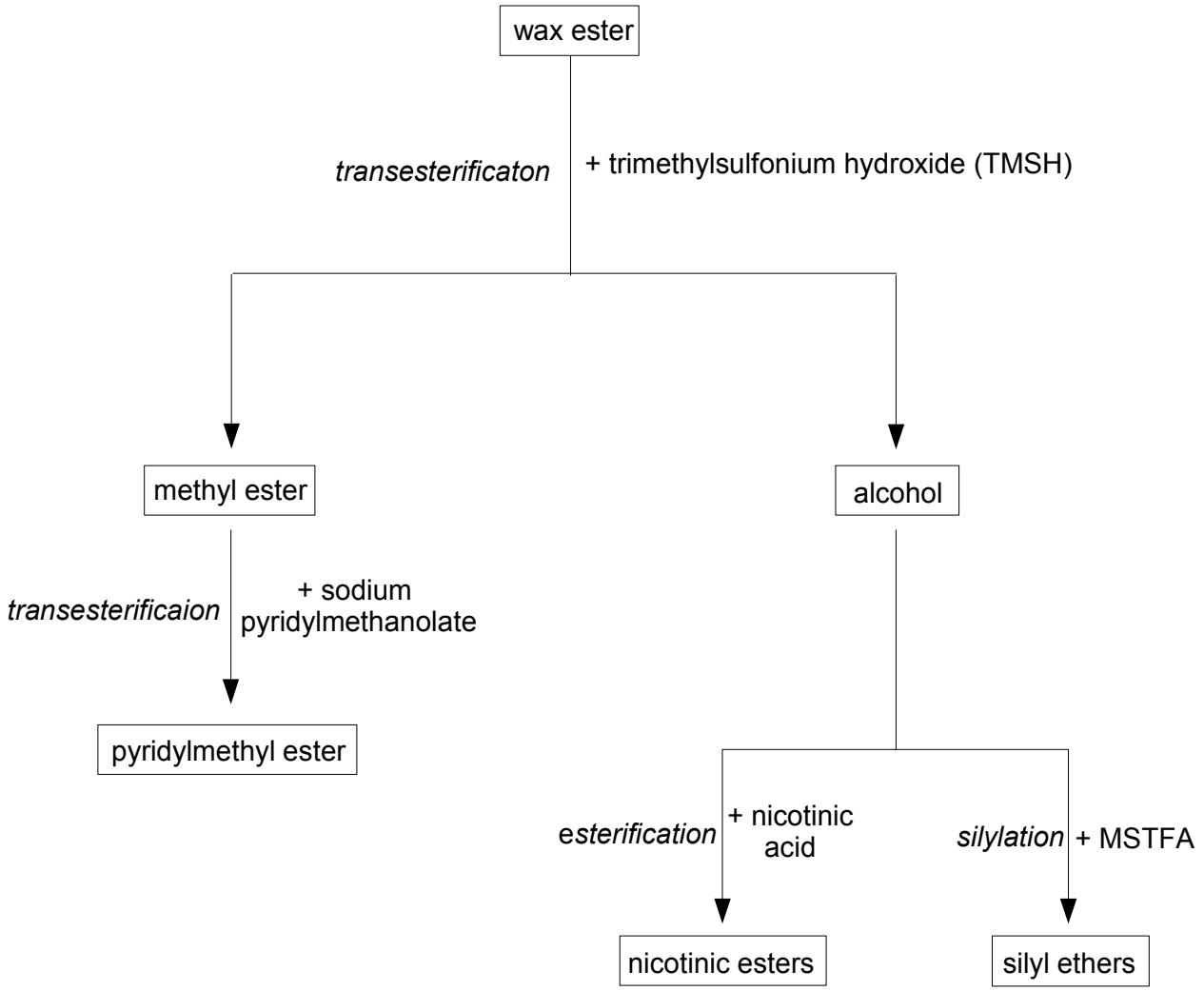

Figure 56: Flow scheme for the analysis of the wax esters from *Argyrodes elevatus*.

Table 7: Compilation of methyl branch increments for methyl esters on a BPX-5-phase [Khorasheh *et al.*, 1989; Schulz, 2001]. FG is an abbreviation for functional group. *I* = increment

	$FG_{MeEster}$	2-Me	internal	(ω-3)	(ω-1)	(ω-2)
Increment (*I*)	336	30	32 - 53	58	60	75

The position of methyl branches of methyl esters at C-2 and C-4 are decuded by characteristic fragment ions [Budzikiewicz, Djerassi, and Williams, 1967]. Methyl esters, branched in 2-position, show the ion pair *m*/*z* = 88, 101 as demonstrated in Figure 56 for methyl 2-methylhexadecanoate.

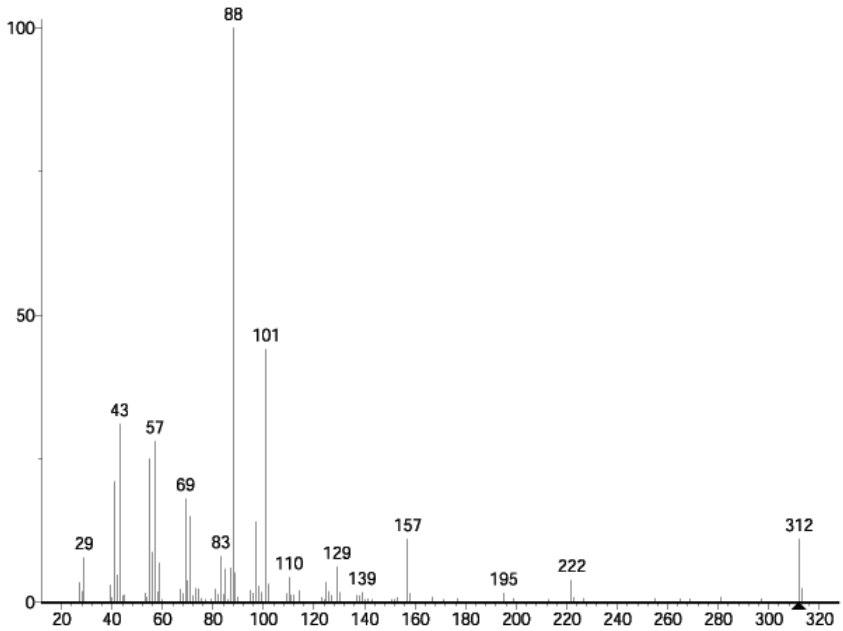

Figure 56: Mass spectrum of methyl 2-methylhexadecanoate [NIST database].

The first ion is formed by a McLafferty rearrangement and the second one is part of a typical ion series in acids and their derivatives with the general formula $[(CH_2)_nCOOMe]^+$. Ions with n = 2, 6, 10, ... are especially preferred and hence the ions *m*/*z* = 101, 157, ... are of higher intensity. The McLafferty ion *m*/*z* = 88 is more abundant than the ion *m*/*z* = 101 in the mass spectra of 2-methylated methyl esters. A methyl group in 4-positon is indicated by the ions *m*/*z* = 74 and 87 formed by the previous discussed fragmentations and in this case, the latter one is more intensive than the McLafferty as shown in Figure 57 for methyl 4-methyloctadecanoate.

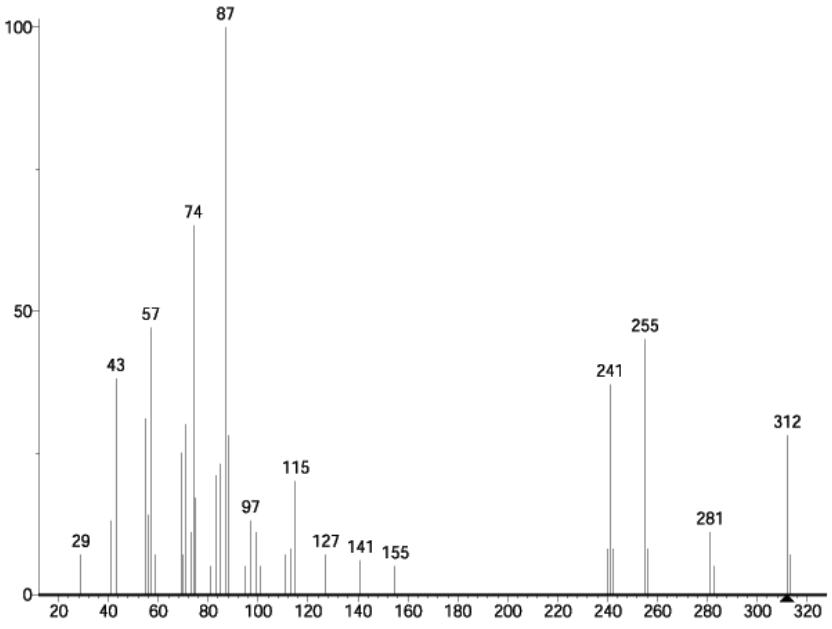

Figure 57: Mass spectrum of methyl 4-methyloctadecanoate [NIST database].

On the other hand, internal and terminal methyl groups are rarely localizable by the analysis of their mass spectra.

The conversion of methyl esters into pyridylmethyl esters by treatment with sodium pyridylmethanolate facilitates the determination of internal and terminal methyl branches because mass spectra of these derivatives are characterized by intensive ions caused by preferred α-cleavages before and after the methyl branch and a gap between them as depicted in Figure 58 for the corresponding derivative of 14-methylhexadecanoate.

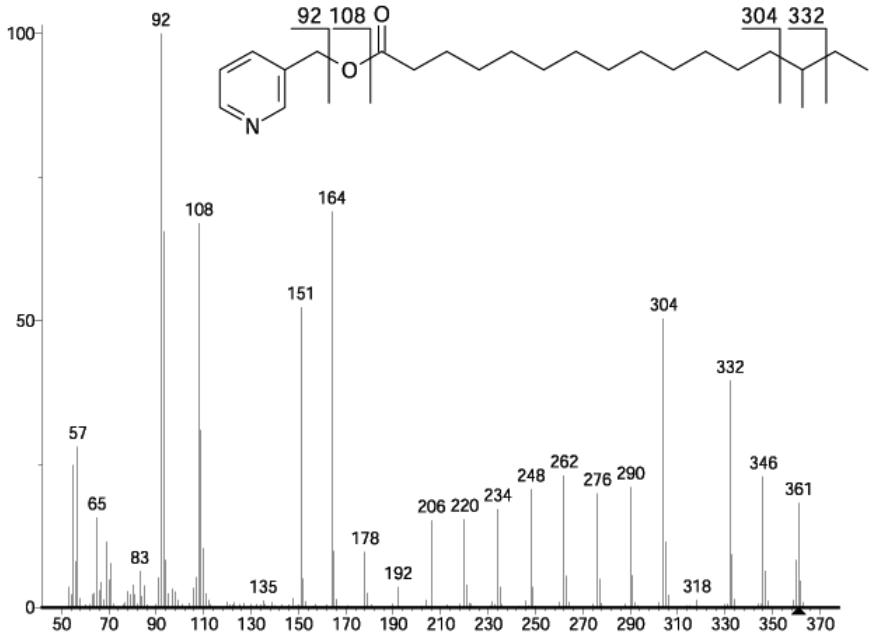

Figure 58: Mass spectrum of the pyridylmethyl ester of 14-methylhexadecanoate [NIST database].

The ion m/z = 92 forms the base peak in these mass spectra and this one evolves by a fragmentation of the C-O-bond in the pyridylhydroxymethyl unit. Further important ions in these mass spectra are displayed by the ions m/z = 108, 151, and 164. The latter ones correspond to the characteristic ions m/z = 74 and 87 in the mass spectra of methyl esters. These ions shift to 165 and 178 amu in the presence of a methyl group in 2-position. The ion m/z = 108 is formed by cleavage of the ester bond. The ions m/z = 304 and 332 and a gap between them indicate the presence of a methyl group in (ω-2)-position.

The alcohols are converted into trimethylsilyl ethers (TMS) and nicotinic esters.

TMS-derivatives are prepared to improve the chromatographic properties (no tailing) compared to the free alcohols. Mass spectra of TMS ethers are characterized by the ion m/z = 75 and the base peak resulting from a loss of a methyl group from the molecular ion. Thus, TMS-ethers are easily recognized in complex blends by ion extraction of the ions m/z = 75 and $[M - 15]^+$. The *RI*s on a BPX-5-phase for a series of TMS ethers of *n*-alcohols were determined to establish an increment

for the TMS-moiety. The results are summarized in Table 8.

Table 8: Increments for the trimethylsilyl moiety in *n*-alcohols on a BPX-5 phase.

Compound	*RI*	Increment
10-ol	1368	368
12-ol	1554	354
14-ol	1749	349
16-ol	1943	343
18-ol	2138	338
20-ol	2329	329

The *RI*s range between 368 for the TMS ether of decanol (10-ol) and 329 for eicosanol (20-ol). These values demonstrate that the increments rely on the number of carbon atoms in the alcohol. The increment decreases with an increasing number of carbon atoms in the alcohol.

Based on these data, a regression analysis was performed (Figure 59). This analysis furnished y = 400 + 96.3x as regression formula and *RI*s of *n*-TMS ethers can be assessed by this formula. For example, a *RI* (y) of 1845 for the TMS ether of pentadecanol (x = 15) can be expected by the utilization of this formula. The impact of methyl groups on the *RI*s of TMS ethers is identical compared to methyl esters and hence position of methyl branches can be predicted with regard to Table 7, Table 8, and the corresponding regression formula.

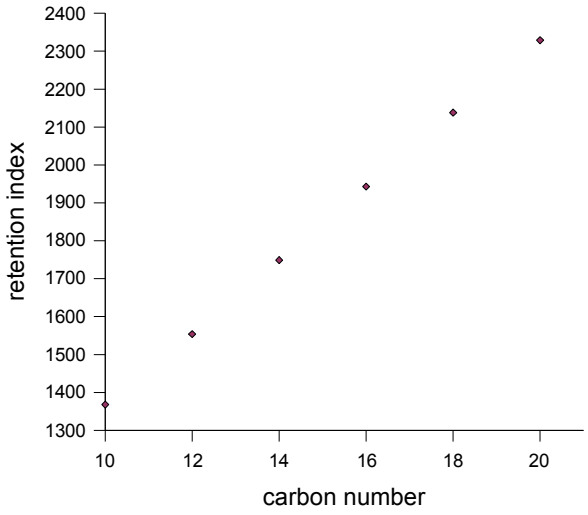

Figure 59: Increment analysis for trimethylsilyl ethers of *n*-alcohols on a BPX-5-phase. The linear plot is described by the regression formula y = 400 + 96.3x.

Conversion of alcohols into nicotinic esters allows the location of methyl branches because the corresponding mass spectra display similar traits as the mass spectra of pyridylmethyl esters. The positions of the methyl branches in the nicotinic ester of 6,10,14-trimethylpentadecanol are emphasized by the ions *m*/*z* = 192, 220, 262, 290, 332, and 360 as well as gaps between them (Figure 60). Furthermore, the ions *m*/*z* = 106 and 124 are characteristic for these mass spectra and they correspond to common fragments in mass spectra of esters.

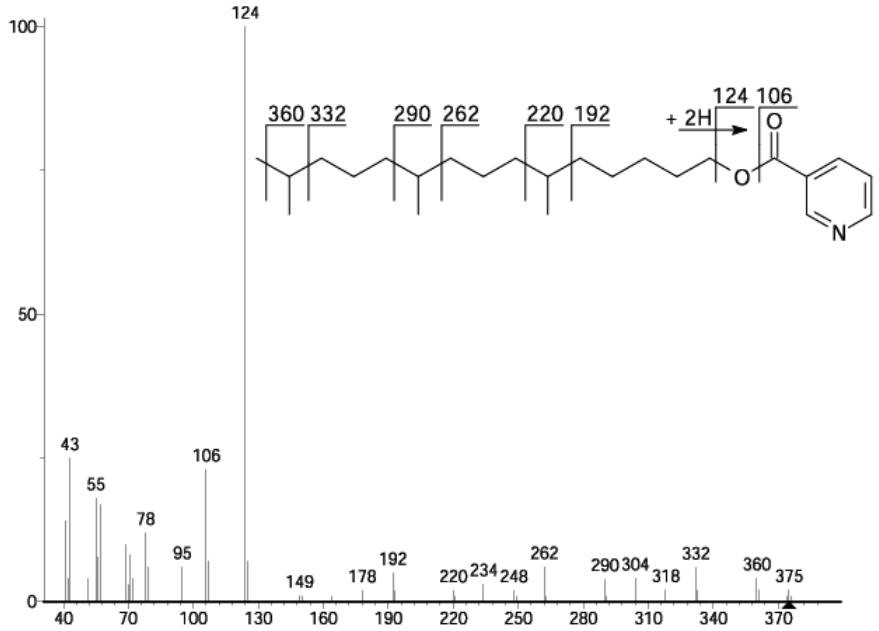

Figure 60: Mass spectrum of the nicotinic ester of 6,10,14-trimethylpentadecanol .

3.1.3.2 Methyl esters and pyridylmethyl esters of *Argyrodes elevatus*

Formation of methyl esters and pyridylmethyl esters furnished derivatives with up to three methyl branches. Structures were proposed based on combination of mass spectral interpretation, comparison between experimental and theoretical *RI*s, and biosynthetic considerations. The composition of the methyl ester blend of the male and female head is shown in Table 9.

The mass spectrum of the corresponding methyl ester of the major wax ester in the male prosoma extract displayed the ions *m*/*z* = 88, 101, and 242 (Figure 61). The latter one indicated the molecular weight and corresponded to a methyl ester with 14 carbon atoms. The first two ions proved the occurrence of a methyl branch at C-2. Furthermore, the absence of additional methyl branches was proposed because this derivative eluted 30 points after methyl tridecanoate (*RI* = 1623); consistent with only one methyl branch at C-2.

This proposal was confirmed by the mass spectrum of the corresponding pyridylmethyl ester in Figure 62. The ion pair *m*/*z* = 165, 178 showed the presence of a methyl branch at C-2 and a complete ion series *m*/*z* = 192, 206, 220, ... as expected for the pyridylmethyl ester of

2-methyltridecanoate.

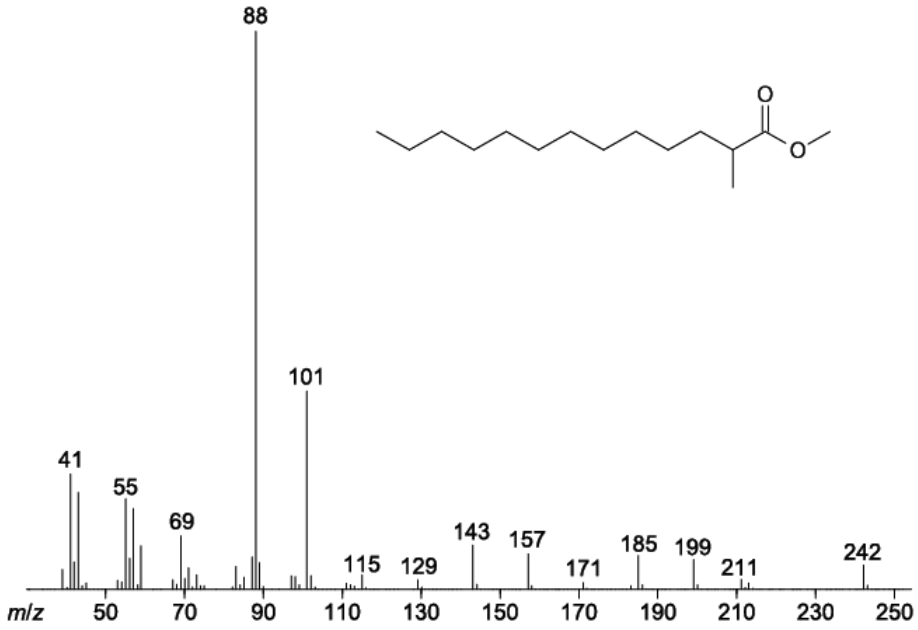

Figure 61: Mass spectrum of methyl 2-methyltridecanoate.

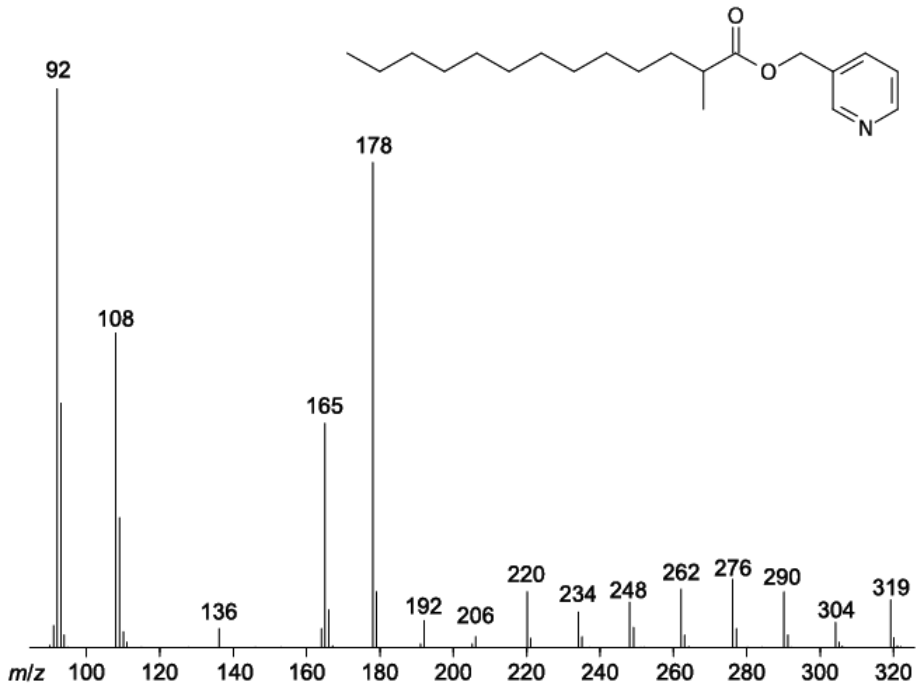

Figure 62: Mass spectrum of the pyridylmethyl ester of 2-methyltridecanoate.

Analogous investigations revealed only two acids present in the four major wax esters of the female head extract. The first two wax esters contained an acid characterized by the mass spectrum of the corresponding methyl ester in Figure 63.

The ion pair *m*/*z* = 88, 101 confirmed the occurrence of a methyl group at C-2 and the ion *m*/*z* =228 was expected for a methyl ester with 13 carbon atoms. Furthermore, an additional methyl branch

was postulated by the consideration of its *RI*. This derivative exhibited a *RI* of 1509, different to the theoretical *RI* of 1553 for methyl 2-methyldodecanoate. Thus, methyl 2,8- or 2,10-dimethylundecanoate were proposed because their theoretical *RI*s were in the range of the observed *RI* of the natural methyl ester. The location of the second methyl branch was elucidated by the mass spectrum of the corresponding pyridylmethyl ester in Figure 64. The ion pair *m*/*z* = 165, 178 indicated the presence of a methyl branch at C-2 and the ions *m*/*z* = 234 and 252 as well as the gap between them an additional methyl branch in position 8. Based on these results, 2,8-dimethylundecanoate was identified as the acid in the first two major wax esters.

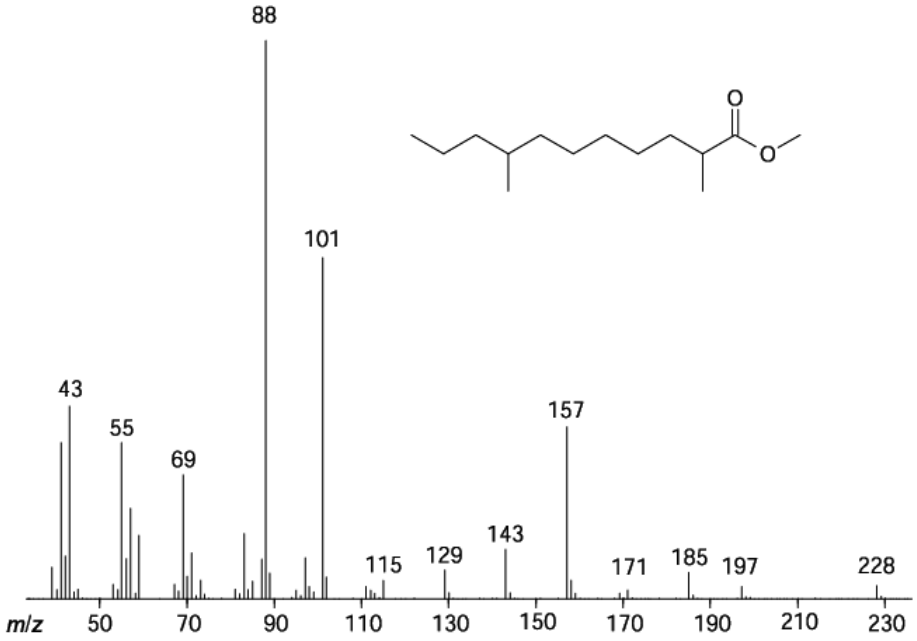

Figure 63: Mass spectrum of methyl 2,8-dimethylundecanoate.

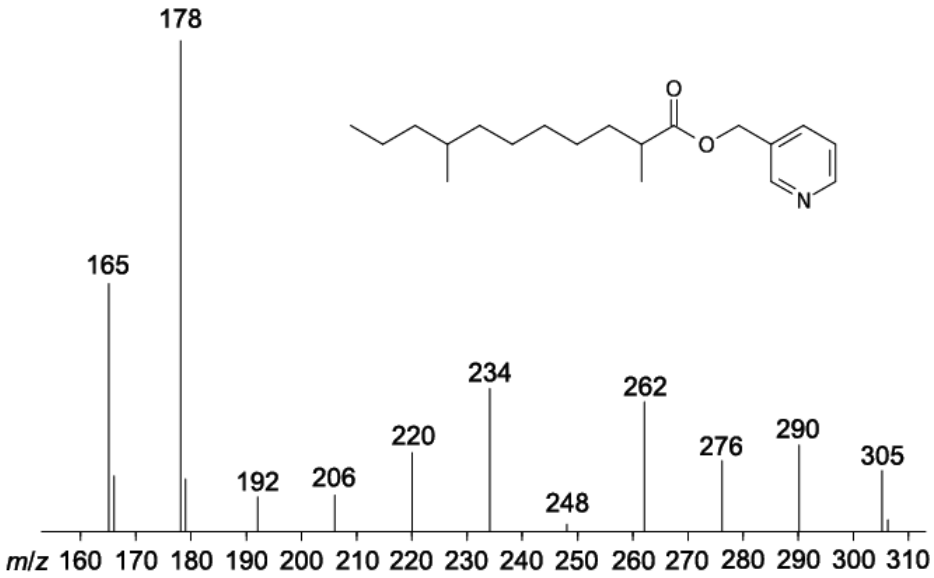

Figure 64: Mass spectrum of the pyridylmethyl ester of 2,8-dimethylundecanoate.

The acid of the third and fourth female wax ester was characterized by the mass spectrum in Figure 65 and a *RI* of 1097. Instead of the ions *m*/*z* = 88 and 101, the ions *m*/*z* = 74 and 87 occurred, thus ruling out a methyl group at C-2. A (ω-1)- or (ω-3)-branched acid with 7 carbon atoms was proposed based on the *RI*, 37 points shorter than that of methyl octanoate. The ion *m*/*z* = 85 was predominant in the mass spectrum of the natural methyl ester, in contrast to a reference mass spectrum of the (ω-1)-branched isomer [NIST database].

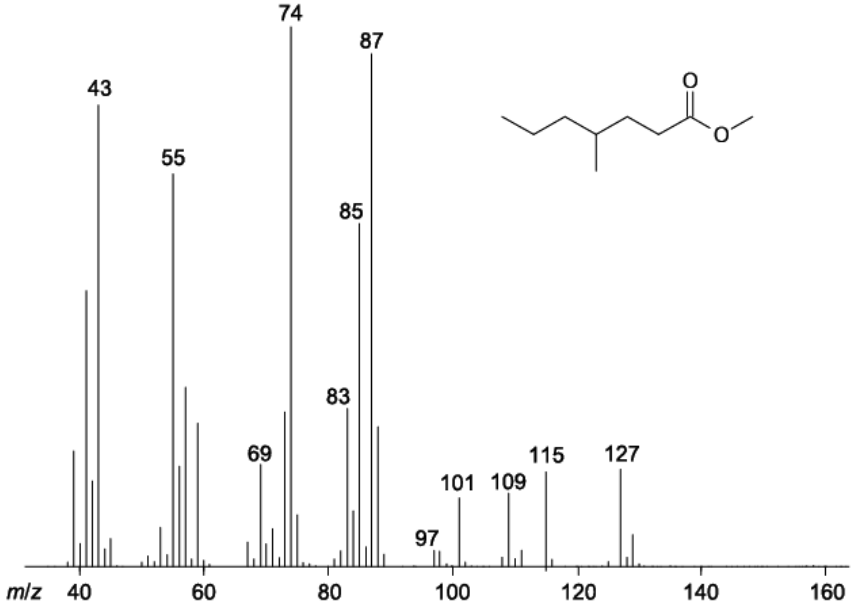

Figure 65: Mass spectrum of methyl 4-methylheptanoate.

Furthermore, other methyl esters with 5 to 15 carbon atoms were identified (Table 9). Corresponding pyridylmethyl esters were not obtained caused by a reduced recovery rate of these derivatives.

Table 9: Methyl esters (Me) obtained after derivatization of head extracts (prosoma) of male and female *Argyrodes elevatus*. Intensities of compounds are given in %. Pro = prosoma.

RI	Compound	Pro, adult fem	Pro, adult mask
-	5:Me	Tr	-
951	6:Me	0.4	-
1006	5Me-6:Me	-	4.1
1016	4Me-6:Me	Tr	-
1039	7:Me	0.2	0.3
1072	2Me-7:Me	Tr	-
1097	4Me-7:Me	3.8	Tr
1134	8:Me	0.2	2.4
1194	7Me-8:Me	Tr	-

RI	Compound	Pro, adult fem	Pro, adult mask
1230	9:Me	5.8	0.5
1281	4Me-9:Me	0.1	-
1291	8Me-9:Me	-	Tr
1321	2,8DiMe-9:Me	Tr	-
1327	10:Me	1.1	1.5
1389	9Me-10:Me	Tr	-
1419	2,9DiMe-10:Me	0.2	Tr
1424	2,8DiMe-10:Me	0.3	-
1426	11:Me	Tr	Tr
1456	2Me-11:Me	0.3	0.1
1481	8Me-11:Me	Tr	-
1487	10Me-11:Me	Tr	-
1509	2,8DiMe-11:Me	83.9	0.3
1516	2,10DiMe-11:Me	-	Tr
1524	12:Me	1.0	0.6
1554	2Me-12:Me	-	0.1
1586	11Me-12:Me	0.3	0.1
1593	10Me-12:Me	Tr	Tr
1600	2,8DiMe-12:Me	Tr	-
1615	2,11DiMe-12:Me	-	0.5
1623	13:Me	Tr	0.1
1653	2Me-13:Me	1.1	87.8
1656	2,8,11TriMe-12:Me	0.1	-
1686	12Me-13:Me	0.1	Tr
1696	2,8DiMe-13:Me	0.3	0.3
1707	2,10DiMe-13:Me	-	0.2
1714	2,12-DiMe-C13:Me	-	Tr
1722	14:Me	1.0	0.8
1751	2Me-14:Me	-	0.2
1850	2Me-15:Me	-	0.1

Methyl branches in even-numbered methyl esters existed internally only in even-numbered- or in (ω-1)- and (ω-2)-position. Internal branched derivatives use acetate as starter unit in their biosynthesis and then the chain is elongated by incorporation of acetate (malonate) or propionate (methylmalonate). Hence, methyl branches are inevitable in even-numbered positions. Derivatives, branched in (ω-1)-position, use another starter unit for the biosynthesis. Here, leucine is transaminated to the corresponding α-keto acid, followed by oxidative decarboxylation to furnish 3-methylbutyric acid and finally, chain extension via acetate (malonate) or propionate (methylmalonate) proceeds. Leucine is replaced by isoleucine in the biosynthesis of the

(ω-2)-branched derivatives but the remaining steps are identical.

Odd-numbered methyl esters contain also methyl branches in even-numbered positions and in the (ω-1)-position. The latter derivatives are analogously formed to the even-numbered (ω-1)-branched derivatives, but valine is used instead of leucine. Internal branched compounds need propionate as starter unit followed by chain extension steps like the mentioned ones above resulting in acids with methyl branches in even-numbered positions.

3.1.3.3 Trimethylsilyl ethers and nicotinic esters of the alcohol parts of the wax esters of *Argyrodes elevatus*

The composition of the blend of TMS ethers of prosoma extracts of male and female *A. argyrodes* is summarized in Table 10.

Table 10: TMS ethers of the wax esters alcohols present in the prosoma (head) extracts of *Argyrodes elevatus* males and females. Amounts of compounds are given in %.

RI	Compound	pro, adult, fem	pro, adult, mask
1460	TMS-11:1-ol	-	0.7
1487	TMS-2Me-11:1-ol	2.0	-
1527	TMS-2,6DiMe-11:1-ol	0.1	-
1540	TMS-2,8-DiMe-11:1-ol	6.9	-
1547	TMS-2,10DiMe-11:1-ol	0.1	-
1555	TMS-12:1-ol	-	Tr
1614	TMS-11Me-12:1-ol	-	0.3
1628	TMS-2,XDiMe-12:1-ol	Tr	-
1642	TMS-2,11DiMe-12:1-ol	0.1	-
1678	TMS-2Me-13:1-ol	0.3	0.3
1720	TMS-2,XDiMe-13:1-ol	1.1	-
1732	TMS-2,10DiMe-13:1-ol	Tr	-
1748	TMS-14:1-ol	0.1	-
1845	TMS-15:1-ol	-	0.3
1941	TMS-16:1-ol	0.3	0.7
2001	TMS-15Me-16:1-ol	1.0	-
2009	TMS-14Me-16:1-ol	0.1	-
2038	TMS-17:1-ol	47.8	0.3
2082	TMS-12Me17:-1-ol	1.9	-
2095	TMS-14Me-17:1-ol	11.8	-
2137	TMS-18:1-ol	2.0	4.2
2231	TMS-19:1-ol	Tr	-
2328	TMS-20:1-ol	8.6	15.0

RI	Compound	pro, adult, fem	pro, adult, mask
2427	TMS-21:1-ol	3.0	-
2524	TMS-22:1-ol	5.0	7.8
2621	TMS-23:1-ol	5.6	7.1
-	TMS-24:1-ol	1.1	32.3
-	TMS-25:1-ol	0.3	2.1
2911	TMS-26:1-ol	0.3	4.1
2970	TMS-25Me-26:1-ol	-	22.8
3068	TMS-26Me-27:1-ol	0.4	1.7
3105	TMS-28:1-ol	0.1	0.3

The female extract comprised derivatives with 11 to 28 carbons and up to two methyl branches.

In contrast to the female extract, the male extract consisted only of *n*-alcohols with a chain length ranging from 11 to 28 carbon atoms. Methyl branches were excluded because the *RI*s of these alcohols were congruent with the theoretical *RI*s of TMS ethers of *n*-alcohols, calculated by the regression formula y = 400 + 96.3x.

The mass spectrum of the TMS ether of undecanol, the preferred alcohol of the male extract, is depicted in Figure 66.

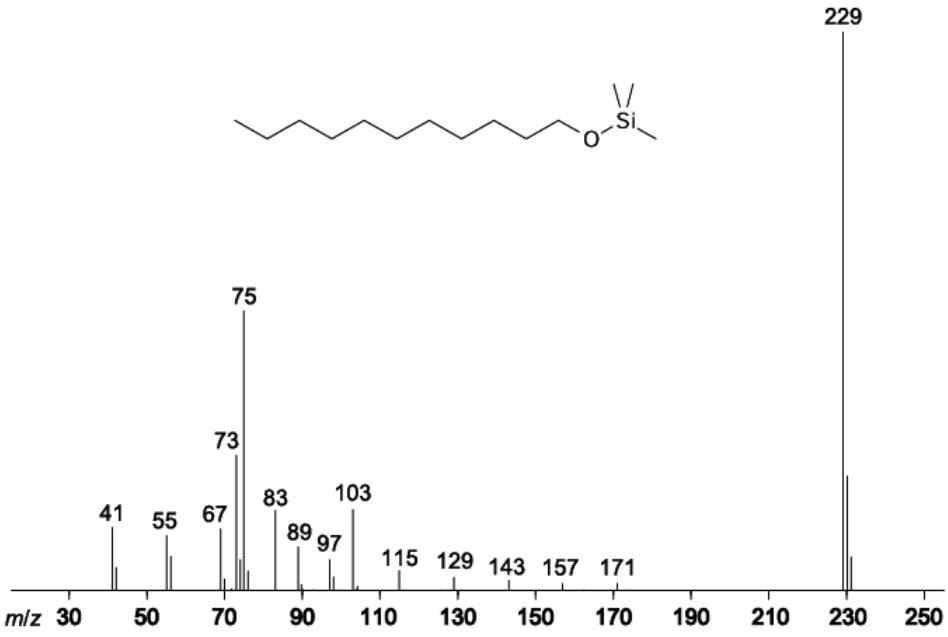

Figure 66: Mass spectrum of the trimethylsilyl ether of 1-undecanol.

The mass spectrum was characterized by the base ion *m*/*z* = 229 and the ions *m*/*z* = 73, 75, and 103, indicative for the TMS ether of 1-undecanol.

This result was confirmed by the corresponding mass spectrum of the nicotinic ester in Figure 67.

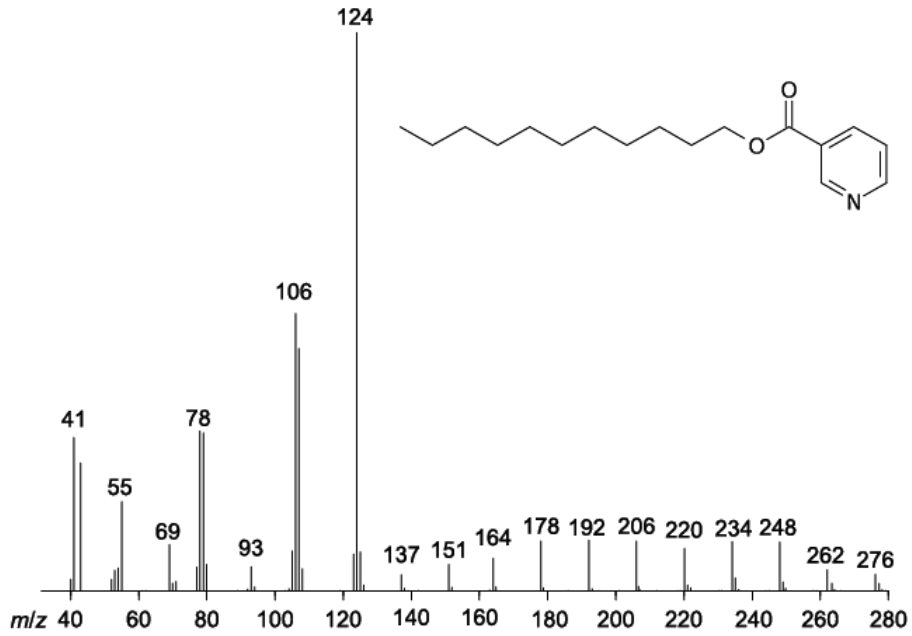

Figure 67: Mass spectrum of nicotinic ester of 1-undecanol.

The molecular ion was indirectly indicated by a loss of a hydrogen from the molecular ion *m*/*z* = 277. The main ion series 164, 178, ... was not interrupted by gaps clarifying the absence of methyl branches in the alkyl chain.

The four major wax esters of the female head extract contained four different alcohols.

The first of these was 2-methylundecanol and the mass spectra of the corresponding TMS ether and nicotinic ester are shown in Figure 68 and 69.

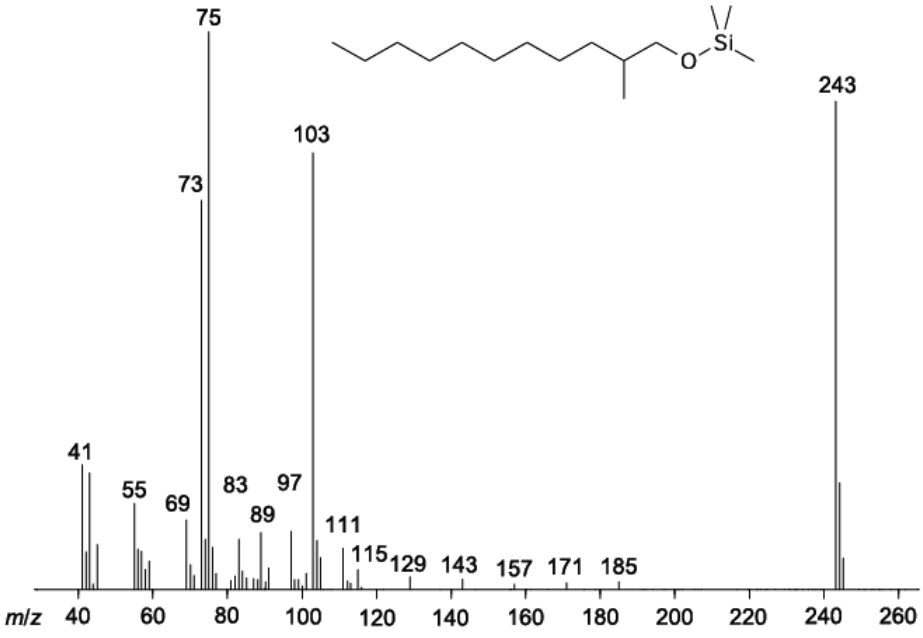

Figure 68: Mass spectrum of the trimethylsilyl ether of 2-methylundecanol.

The ion *m*/*z* = 243 $[M-15]^+$ showed the presence of an alcohol with 12 carbon atoms. The *RI* of 1487 was not in agreement with the theoretical *RI* of 1556 for the TMS ether of dodecanol. This compound eluted 30 points later than the TMS ether of undecanol and hence, the presence of a methyl branch in 2-position was postulated. This assumption was supported by the increased intensity of the ions *m*/*z* = 75 and 103 compared to the mass spectra of TMS ethers of *n*-alcohols, explainable by a preferred cleavage in α-position to the TMS moiety, caused by the presence of a methyl branch at C-2.

The corresponding nicotinic ester confirmed this proposal because a predominant ion *m*/*z* = 164 was observed in its mass spectrum in accordance to a methyl branch at C-2 triggered by a cleavage after the methyl branch. This ion was even-numbered contrary to the other ions in the neighbourhood. A lack of gaps in the main ion series with the ions 192, 206, ... indicated the absence of additional methyl branches.

Figure 69: Mass spectrum of nicotinic ester of 2-methylundecanol.

The corresponding mass spectrum of the TMS ether of the alcohol of the second major female wax ester is illustrated in Figure 70. The enhanced intensity of the ions *m*/*z* = 75 and 103 proved also a methyl branch in 2-position. The ion *m*/*z* = 257 demonstrated that the alcohol consisted of 13 carbon atoms. The derivative was characterized by a *RI* of 1540 and this value was 53 points larger than the *RI* of the previous discussed derivative. Hence, an additional methyl branch in (ω-3)-position was postulated because an increment of 53 points was thought to be evoked by a methyl branch in this position, analogously to 2,8-dimethylundecanoate before.

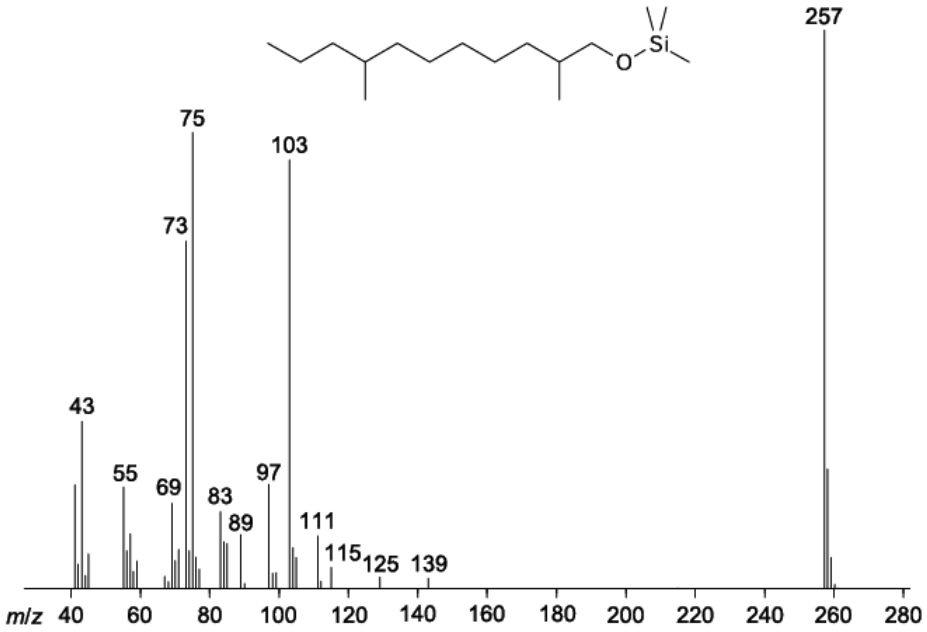

Figure 70: Mass spectrum of the trimethylsilyl ether of 2,8-dimethylundecanol.

This proposal was supported by the mass spectrum of the corresponding nicotinic ester in Figure 71. The methyl branch at 2-positon was indicated by a slight enhanced intensity of the ion *m*/*z* = 164 compared with the neighbouring ions. The ion series 192, 206, ... was interrupted between the ions *m*/*z* = 234 and 262. This result pointed to the occurrence of an additional methyl branch at C-8 and 2,8-dimethylundecanol as structure.

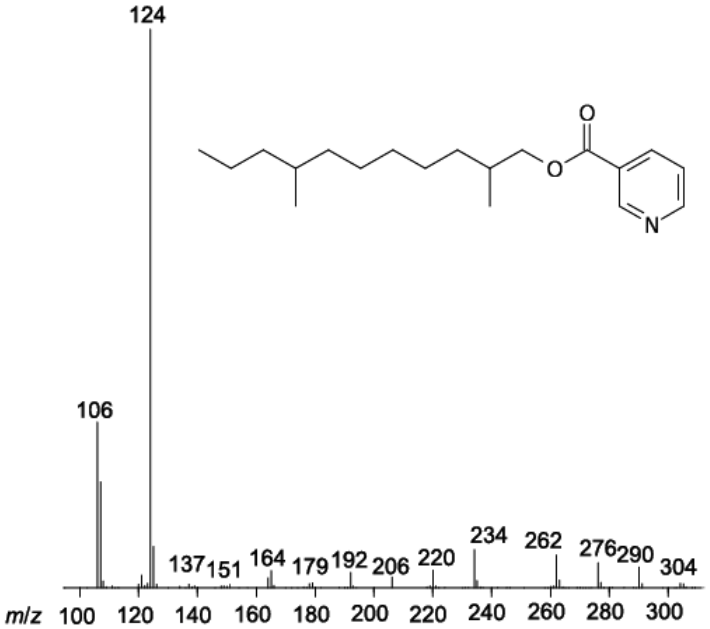

Figure 71: Mass spectrum of the nicotinic ester of 2,8-dimethylundecanol.

The alcohol of the third major female wax ester was identified by similar reasoning as

1-heptadecanol (Figures 72 and 73).

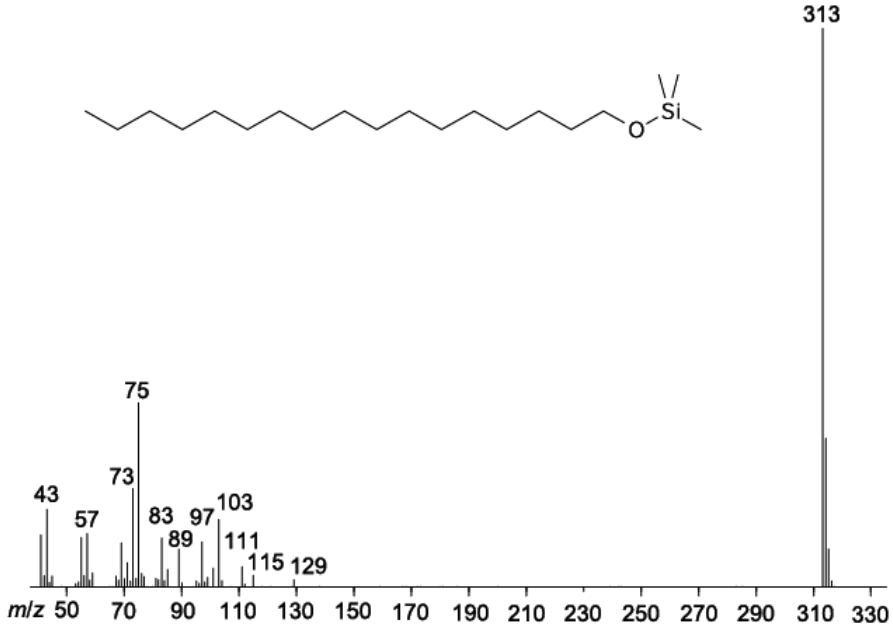

Figure 72: Mass spectrum of the trimethylsilyl ether of 1-heptadecanol.

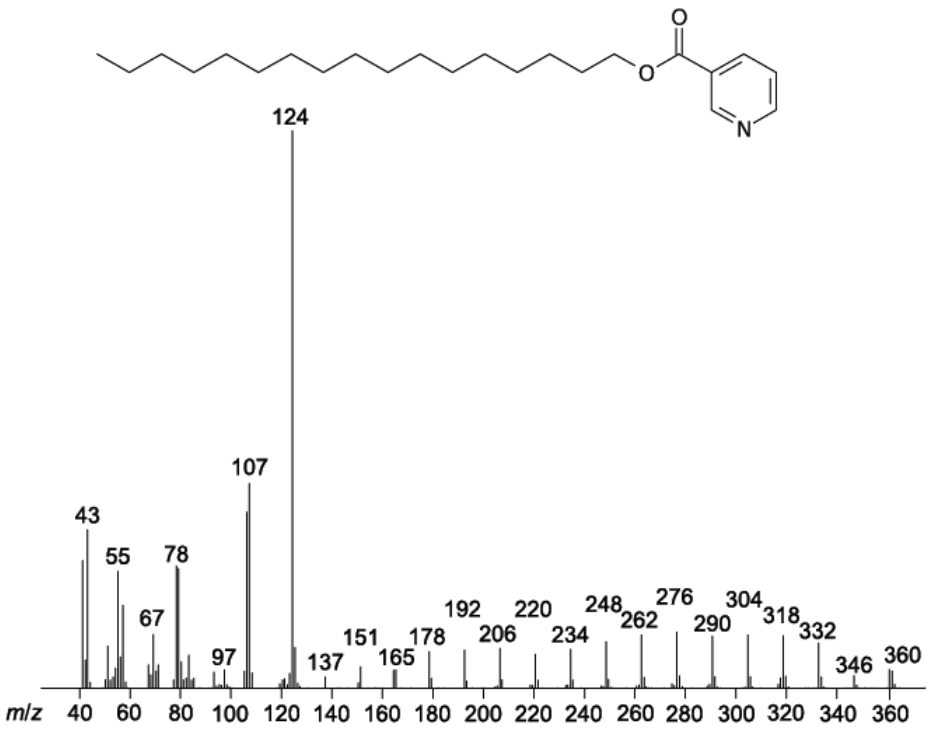

Figure 73: Mass spectrum of the nicotinic ester of 1-heptadecanol.

The mass spectrum of the fourth TMS ether, containing 18 carbon atoms, is shown in Figure 74. The minor intensity of the ions *m*/*z* = 75 and 103 excluded a methyl branch at C-2. The *RI* of 2095 was measured and this value was 47 points larger than the value for the TMS ether of 1-heptadecanol. This result indicated the occurrence of a methyl branch in the alkyl chain.

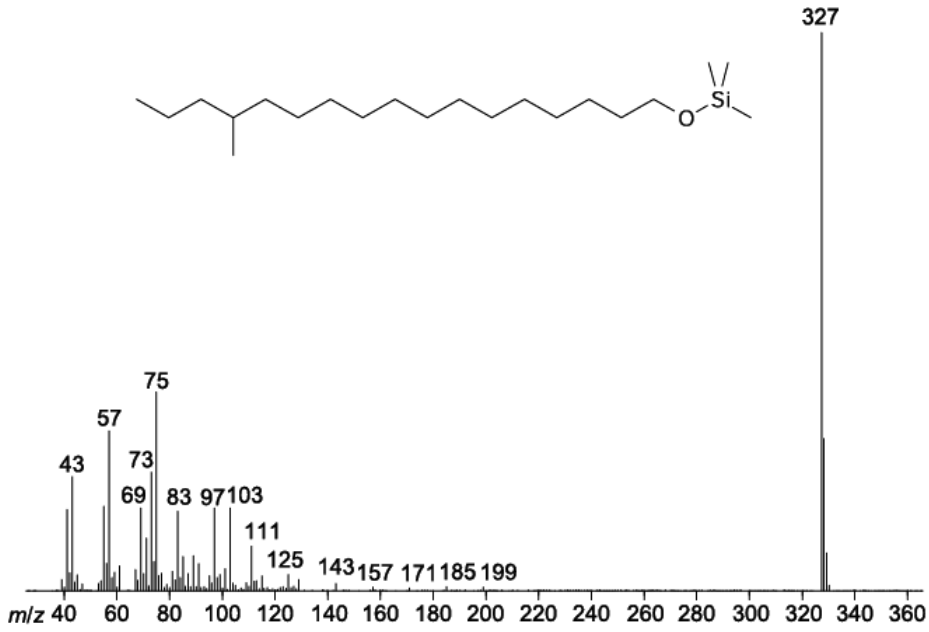

Figure 74: Mass spectrum of the trimethylsilyl ether of 14-methylheptadecanol.

The exact position of the methyl group was determined by the mass spectrum of the corresponding nicotinic ester in Figure 75. The increased intensity of the ions *m*/*z* = 304 and 332 and the gap between them revealed a methyl branch in the (ω-3)-position and 14-methylheptadecanol as alcohol.

Figure 75: Mass spectrum of the nicotinic ester of 14-methylheptadecanol.

3.1.3.4 Wax esters of the head extracts of *Argyrodes elevatus*

Table 10 compiles the identified wax esters in the male and female head extract of *A. elevatus*. In general, acids with ≤ 10 carbon atoms are esterified with alcohols bearing ≥ 16 carbon atoms in the chain. On the other hand, acids with more than 10 carbon atoms are esterified with alcohols of a similar chain length. Recently, all major wax esters were synthesized in our group and the data of the synthetic references were in accordance with the data shown here [Chinta, 2007].

Table 10: Wax esters identified in the male and female head extract of *Argyrodes elevatus*. Intensities of compounds are given in %.

RI	Compound	fem	mask
2379	2Me11Ac:11OH	-	0.4
2390	5Ac:17OH	0.3	-
2416	2Ac:20OH	0.2	Tr
2422	Ac(197, 215):12OH	0.3	-
2431	2,8DiMe11Ac:11OH	-	0.4
2437	2,10DiMe11Ac:11OH	-	0.2
2440	4Me7Ac:15OH	0.2	-
2441	14Ac:10OH	-	„
2446	5Ac:14Me17OH	0.3	-
2452	6Ac:15Me16OH	0.5	-
2461	2,8DiMe11Ac:2Me-11OH	11.3	0.1
2476	2Me12Ac:11OH; 2Me13Ac:10OH	-	0.2
2494	2,8DiMe11Ac:2,6DiMe11OH	1.0	-
2514	2,8DiMe11Ac:2,8DiMe11OH	28.0	0.2
2538	2,11DiMe12Ac:11OH	-	5.0
2539	4Me7Ac:16OH	0.4	-
2559	7Ac:15Me16OH	1.2	-
2581	2Me13Ac:11OH	-	76.0
2584	7Ac:17OH	0.6	-
2601	8Ac:17OH, 7Ac:18OH	1.4	-
2604	Ac(211, 229):11OH	-	0.2
2614	2,8DiMe13Ac:11OH	-	0.5
2616	8Ac:17OH, 7Ac:18OH	0.8	-
2629	2,10DiMe13Ac:11OH	-	0.3
2636	12Me13Ac:12OH	-	0.5
2642	4Me7Ac:17OH	26.2	0.2
2655	2,8DiMe11Ac:2Me13OH	1.1	0.2
2673	2Me13Ac:12OH	-	0.9
2684	4Me7Ac:12Me17OH	2.1	-
2697	4Me7Ac:14Me17OH	10.3	-

RI	Compound	fem	mask
2741	4Me7Ac:18OH	Tr	-
2772	2Me13Ac:13OH	-	-
2779	9Ac:17OH	Tr	-
2793	5Ac:21OH	Tr	-
2799	2Me13Ac:2Me13OH	-	-
2822	10Ac:17OH	Tr	-
2827	2,11DiMe12Ac:14OH	-	-
2835	9Ac:14Me17OH	Tr	-
2842	4Me-7Ac:19OH	Tr	-
3027	2,8DiMe11Ac:17OH; 2,10DiMe12Ac:16OH	Tr	Tr
3080	9Ac:20OH	Tr	-
3095	5Ac:24OH	Tr	-
3181	9Ac:21OH	0.6	-
3257	7Ac:24OH	-	1.5
3327	5Me6Ac:25OH	-	Tr
3360	7Ac:25OH	-	0.3
3383	9Ac:23OH	0.2	-
	7Ac:27OH	-	0.5

3.2 New methoxyalkanes in spiders: 2-methoxyalkanes from the spider *Diplocephalus permixtus*

3.2.1 Evolution of peculiar head structures in Erigoninae males

Males of *Diplocephalus permixtus* are interesting because they are characterized by an unique head structure [Schaible and Gack, 1987]. In the case of *D. latifrons*, protuberances and holes occur in the head region and this structure is porous and associated with epidermal glands. Ducts from these glands lead to these pores and their secretion is released by them to the surface of the head structure. During mating, this morphological structure is touched by mouthparts of the female and the released secretion is ingested by female saliva and finally taken up. Furthermore, two male morphs exist in the closely related species *Oedothorax gibbosus* [Vanacker *et al.*, 2003]. The gibbosus morph is featured by a hairy groove which is absent in the tuberosus morph and courtship as well as mating are longer than in the tuberosus morph. The hairy groove releases a secretion similar to *D. latifrons*. Courtships are displayed with conspecific females and males as well as with males of *O. fuscus*. This secretion is sequestered by the female during mating and also by intra- or interspecific males. This secretion serves as nuptial gift during mating with conspecific females. On the other hand, homosexual interactions are directed to the robbing of this secretion.

3.2.2 Occurrence of 1- and 2-methoxyalkanes in *Diplocephalus permixtus*

Female and male prosoma extracts of the spider *D. permixtus* were investigated by GC-MS and besides 1-methoxyalkanes, 2-methoxyalkanes also occurred in these extracts. The latter ones were unknown before. Male and female cuticle differed in their composition only quantitatively. In this section, the results of the analysis of the female prosoma extract are described. 1-Methoxyalkanes were detected in these extracts by the ion m/z = 45, while the ion m/z = 59 was useful for the detection of 2-methoxyalkanes. Figure 76 shows an ion extract of the ions m/z = 45 and 59 from a prosoma extract of female *D. permixtus*. The analysis of this extract was troublesome because coelution of 1- and 2-methoxyalkanes provided mixed mass spectra.

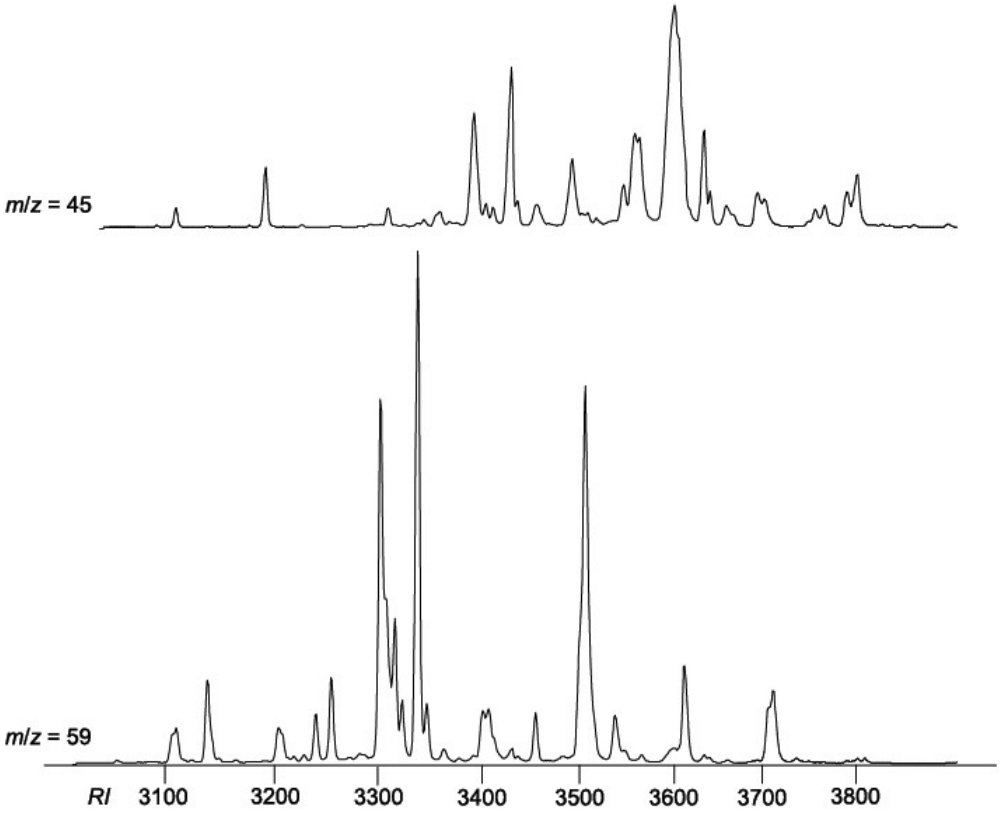

Figure 76: Ion extract (m/z = 45, m/z = 59) of a total ion chromatogram from a prosoma extract of *Diplocephalus permixtus*.

3.2.2.1 Analysis of 1-methoxyalkanes

The ion m/z = 45 is formed by a cleavage in α-position to the methoxy unit. The mass spectra of 1-methoxyalkanes resemble those of 1-alcohols and both feature an elimination of water and methanol, respectively. Subsequently, a cleavage of ethene follows in both cases. Thus, mass spectra of 1-methoxyalkanes contain the general ions $[M - 32]^{+\cdot}$ and $[M - 32 - 28]^{+\cdot}$ and these ions, though occurring only in small intensities, are valuable for the determination of the molecular weight of these derivatives. The remaining part of the mass spectra, except of the ion m/z = 45, is

similar to a common mass spectrum of an alkene. Figure 77 shows the mass spectrum of 1-methoxy-26-methylnonacosane (26Me-29:1-OMe) as an example. In this case, the previously discussed ions correspond to the ions *m*/*z* = 392 and 420.

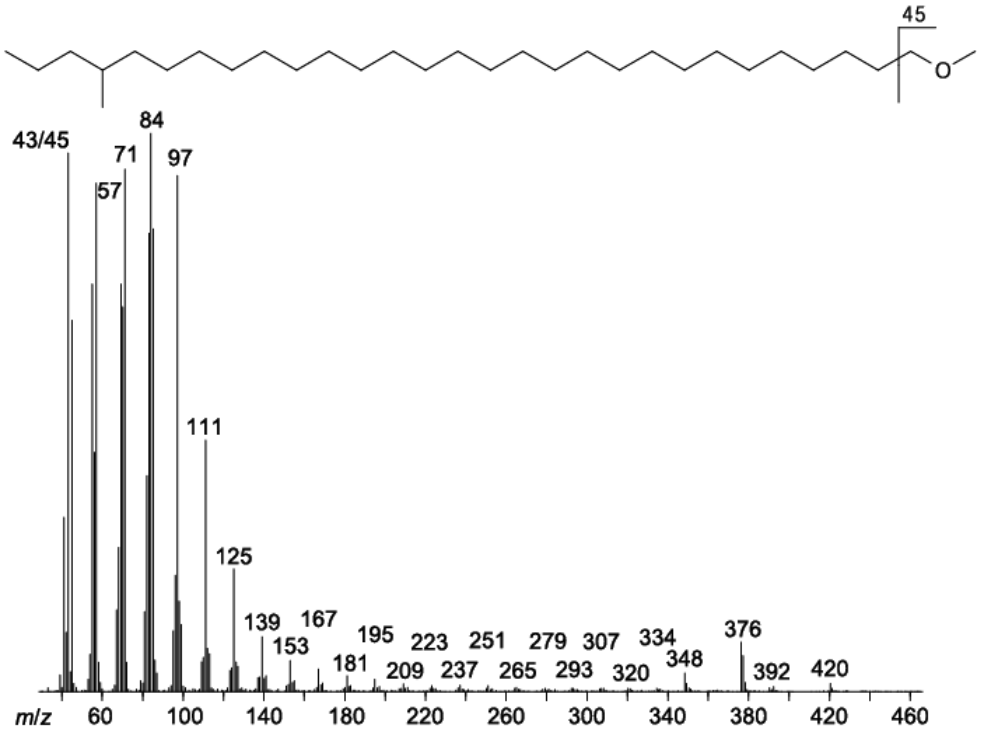

Figure 77: Mass spectrum of 1-methoxy-26-methylnonacosane.

The ions *m*/*z* = 84, 348/349, and 376/377 in this mass spectrum are important because they indicate the existence of a methyl branch in (ω-3)-position by the general ions $[M - 43/44]^+$ and $[M - 71/72]^+$. The latter ones are caused by a preferred cleavage in α-position to the branch. Corresponding ions are also visible in the mass spectra of (ω-2)-branched derivatives. In this case, the ion *m*/*z* = 70 is significant and furthermore, the ions $[M - 29/30]^+$ and $[M - 57/58]^+$ are present.
In contrast, positions of internal methyl groups can not be elucidated from the mass spectra because these fragmentations provide ions which are not easily to explain [Schulz, 2001].
However, 1-methoxyalkanes contain often methyl branches and methoxyalkanes with up to four methyl groups were identified by comparison of their *RI*s with those of unbranched isomers. An increment of 232 points was described in literature for the 1-methoxy unit [Schulz and Toft, 1993]. Two derivatization methods are available allowing the elucidation of internal methyl branches and those near to the methoxy unit and both were applied, here [Schulz and Toft (1993)].
Oxidation of 1-methoxyalkanes to methyl esters by reaction with ruthenium tetroxide allows the determination of methyl branches at C-2- or C-4 [Carlsen *et al.*, 1981; Schulz and Toft, 1993]. The features of mass spectra of 2-methyl and 4-methyl methyl esters were discussed in detail in section

3.1.3.1 .

The mass spectrum of a tetramethylated methyl ester is shown as an example in Figure 78.

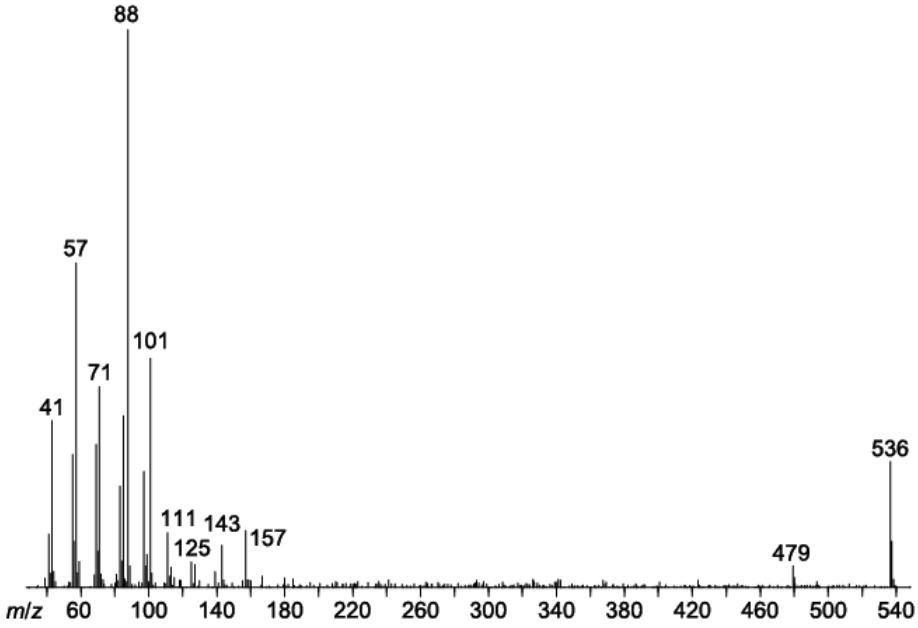

Figure 78: Mass spectrum of a tetramethylated methyl ester with a methyl branch in 2-position.

The ions *m*/*z* = 88 and 101 demonstrated the presence of a methyl group at C-2. The *RI* of this ester was 3588 and only a tetramethylated ester was in agreement with this experimental *RI*. Theoretical considerations led to the postulation of a derivative with a methyl group at C-2, two internal methyl branches, and a fourth methyl group at the alkyl-end. A chain length of 31 carbon atoms with 3100 points, 336 points for the methyl ester increment, 39 points for the methyl group at C-2, 60 points for two internal methyl groups, and 50-55 points for a methyl branch near the ω-end result in a *RI* between 3585-3590, similar to the experimental value.

Conversion of methyl ethers into the corresponding alkyl cyanides allows the determination of internal methyl branches because these appear as outstanding ions in the mass spectra of branched alkyl cyanides [Schulz, 1997a]. Alkyl cyanides are obtained by the cleavage of the methoxy unit with trimethylsilyl iodide (TMSI) under formation of the corresponding iodides followed by treatment with tetraethylammonium cyanide to furnish the alkyl cyanides. Position of internal methyl branches can be elucidated because these derivatives fragment favourable in α-position to the methyl branches. Thereby, the positive charge remains in the nitrogen-containing fragment. Elimination of HCN, providing a mass spectrum similar to alkenes and thus unsuitable for the deduction of branch positions, plays only a subordinate role in these mass spectra. Based on the *RI*s of alkyl cyanides, an increment between 450 and 460, depending on the number of carbons in the straight chain, was deduced [Schulz, 2001]. The mass spectrum of

2,18,20,26-tetramethylhentriacontane-1-carbonitrile (2,18,20,26TetraMe-31:1-CN) is illustrated in Figure 79 as an example.

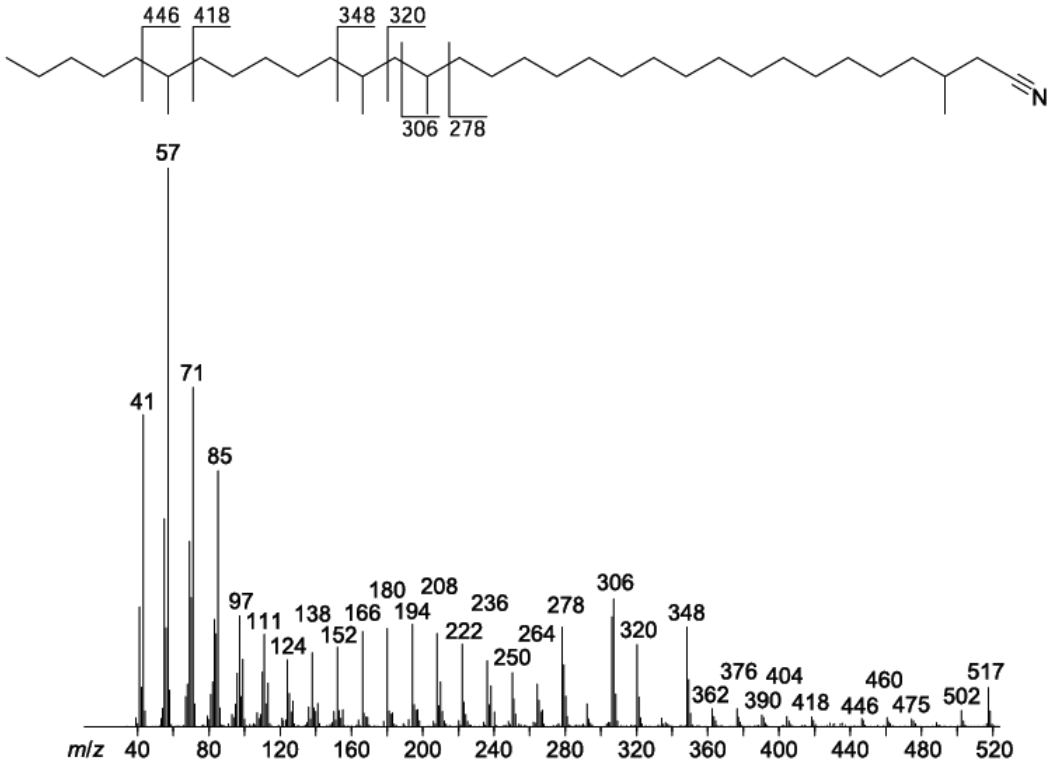

Figure 79: Mass spectrum of 2,18,20,26-tetramethylhentriacontane-1-carbonitrile.

Internal methyl branches in position 18, 20 and 26 were elucidated by the ion pairs *m*/*z* = 278, 306; 320, 348, and 418, 446. Furthermore, the *RI*s for the corresponding 1-methoxyalkanes (3496), the methyl ester (3588), and for the carbonitrile (3729) supported this structural proposal.

The biosynthesis of these derivatives can be rationalized by the use of acetate as an starter unit, in the case of even-numbered 1-methoxyalkanes, and chain elongation with propionate (methylmalonate) to form 2-methylbutyric acid as intermediate. Chain extension proceeds via incorporation of acetate (malonate) or in the case of additional methyl branches in the chain via propionate (methylmalonate). The acid function is then reduced to the corresponding alcohol which is subsequently methylated by S-adenosyl methionine (SAM) to furnish the methyl ether. In contrast, derivatives with a methyl group in (ω-3)-position depend on propionate as starter unit, followed by addition of propionate (methylmalonate) and then, chain extension, reduction, and methylation of the head moiety finish their formation.

3.2.2.2 Analysis of 2-methoxyalkanes

The ion *m*/*z* = 59 in 2-methoxyalkanes is analogously evoked by a cleavage in α-position to the methoxy moiety as the ion *m/z* = 45 in 1-methoxyalkanes. Initially, it was unclear if these

derivatives with the ion *m*/*z* = 59 were 2-methoxyalkanes or ethoxyalkanes. But a comparison between mass spectra of the natural compounds and 2-methoxyhenicosane (21:2-OMe) proved the presence of a 2-methoxy unit in the natural compounds. The reference compound 21:2-OMe **58** was synthesized according to Figure 80. Eicosanal **56** was treated with methylmagnesium chloride to furnish 2-henicosanol **57** in 90% yield. Then, methylation with methyl iodide under reflux in the presence of sodium hydride provided **58** in 95% yield.

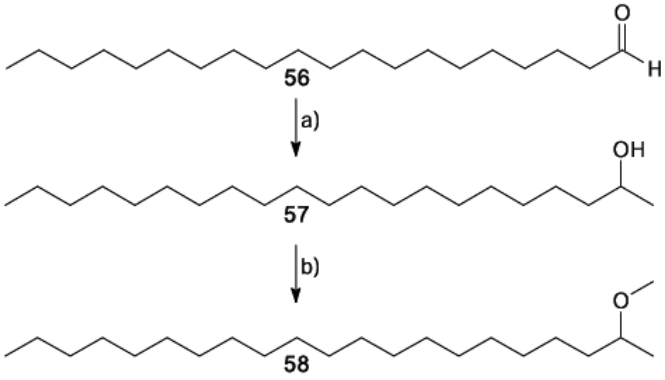

Figure 80: Synthesis of 2-methoxyhenicosane. a) MeMgCl (3M in THF), THF, 48h rt, 90%; b) NaH (80% in mineral oil), THF, MeI, 3.5h reflux, 95%.

The existence of the 2-methoxy unit was clarified by the dominant appearance of the ion *m*/*z* = 59 in the mass spectrum of 21:2-OMe and in those of the natural compounds as shown in Figure 81 and 82. In contrast, the fragmentation pattern of 1-ethoxyalkanes resembles that of 1-methoxyalkanes with the exception that the ion *m*/*z* = 45 is replaced by the ion *m*/*z* = 59.

Ions other than *m*/*z* = 59 are just of subordinate importance in mass spectra of 2-methoxyalkanes. Small ions in the upper mass region, *m*/*z* = 406, 434, and 451, were useful for the deduction of the molecular weight of 466 amu. The ion *m*/*z* = 451 was evoked by a loss of a methyl group from the molecular ion. The ions *m*/*z* = 406 and 434 are putatively formed by a loss of methanol followed by a loss of ethene.

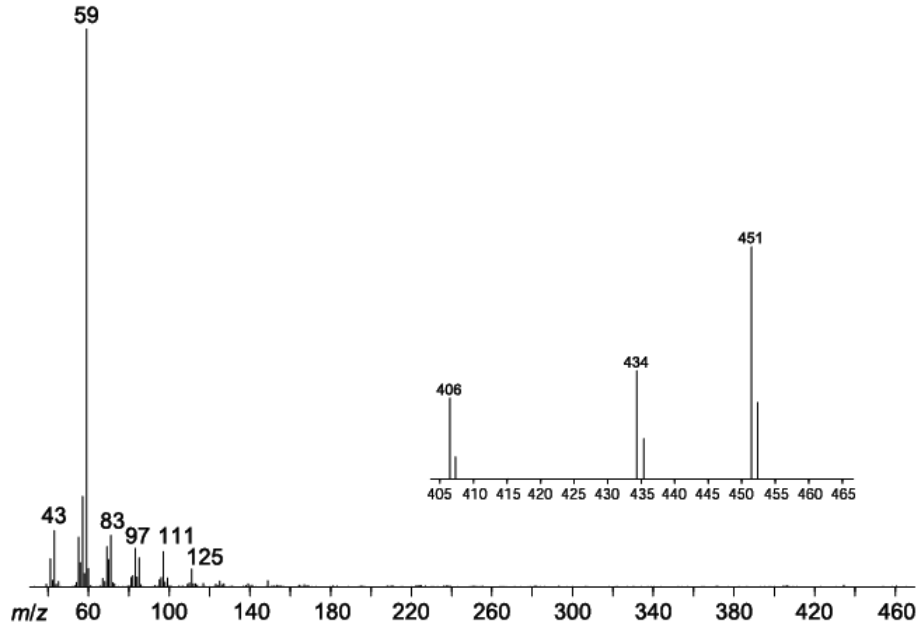

Figure 81: Mass spectrum of a 2-methoxyalkane from the female prosoma extract of *Diplocephalus permixtus*.

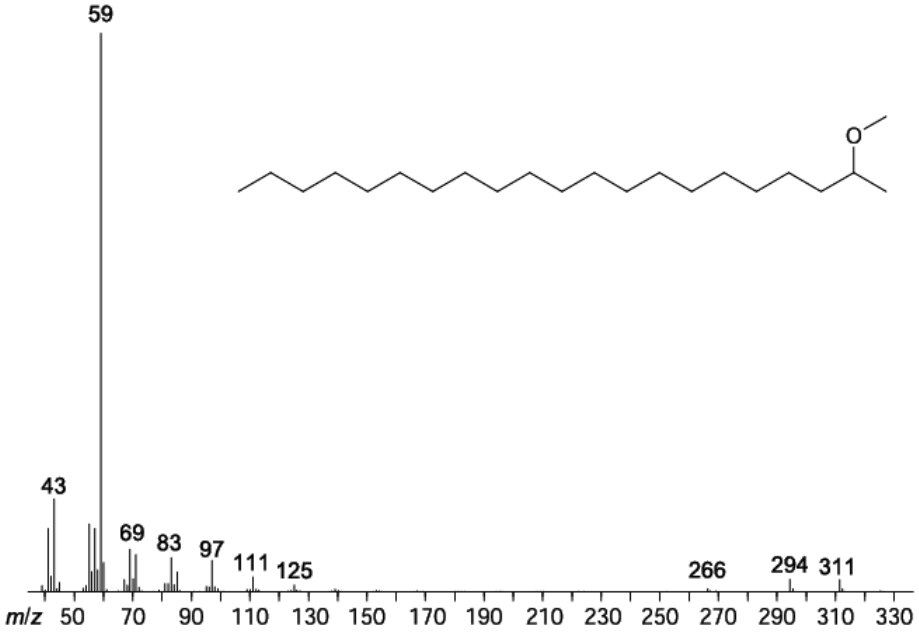

Figure 82: Mass spectrum of 2-methoxyhenicosane.

The natural 2-methoxyalkanes were branched as demonstrated by comparison of *RI*s between natural compounds and unbranched reference compounds. An increment for the 2-methoxy unit of 180 ± 2 points was determined on a BPX-5-phase as shown in Table 11. A blend of 2-methoxyalkanes between 11 and 21 carbon was prepared in a microreaction for this purpose. The analysis demonstrated that the *RI*s of the natural compounds were smaller than those of the unbranched isomers.

Table 11: Determination of the increment of a 2-methoxy unit on a BPX-5-phase. MV = mean value, SD = standard deviation.

Compound	*RI*	Increment
11:2-OMe	1282	182
13:2-OMe	1481	181
18:2-OMe	1977	177
19:2-OMe	2078	178
21:2-OMe	2283	183
	MV	180
	SD	2

The positions of these methyl branches were also investigated by transformation into alkyl cyanides. Unfortunately, this conversion does not proceed quantitatively, caused by elimination of HI during the formation of the alkyl iodides as described in the literature [Carey and Sundberg, 1995]. Nevertheless, this method allowed the determination of methyl branches in most of the 2-methoxyalkanes. The increment for a 2-carbonitrile unit was established by the determination of *RI*s for a carbonitrile blend with chain lengths between 11 and 21 carbon atoms. The values are summarized in Table 12. The values depended on the chain length as found for the corresponding 1-carbonitriles.

Table 12: Increment for the 2-carbonitrile unit on a BPX-5-phase.

Compound	*RI*	Increment
11:2-CN	1428	328
13:2-CN	1635	335
18:2-CN	2146	346
19:2-CN	2244	344
21:2-CN	2454	354

The regression analysis in Figure 83 was performed to predict the *RI* for 2-carbonitriles in dependence of the number of carbon atoms in the straight chain, furnishing the equation y = 303 + 102.33*x (y corresponds to the *RI,* x to the number of carbon atoms in the alkyl chain). Structural proposals were checked by applying this equation after mass spectral analysis of these derivatives and calculated *RI*s and experimental values of these derivatives were very similar.

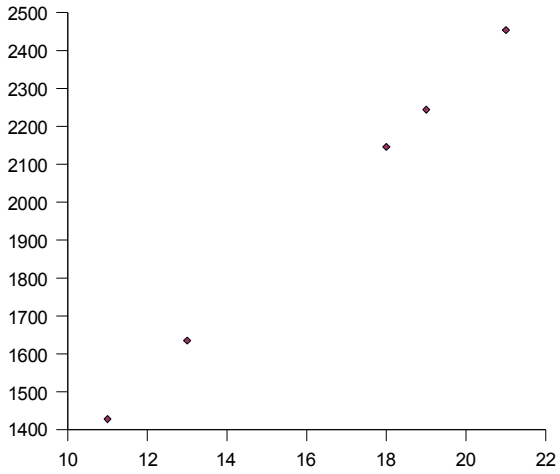

Figure 83: Determination of an increment for a 2-carbonitrile moiety on a BPX-5-phase. Regression analysis furnished the equation y = 303 + 102.33*x for the calculation of the *RI* in dependence of the number of carbon atoms.

In general, 1-methoxyalkanes predominated and contributed 90.8% to the TIC, the remainder being 2-methoxyalkanes (9.2%).

In contrast to 1-methoxyalkanes with up to four methyl branches, 2-methoxyalkanes with one or three methyl branches were identified by mass spectral analysis of the corresponding alkyl cyanides. This analysis was difficult because the compounds occurred only in traces in the extracts and the corresponding mass spectra were characterized by large background noise. Coelution of 1-carbonitriles and 2-carbonitriles posed another problem. However, mass spectral analysis demonstrated that the methyl branches were always in even-numbered positions. Monomethylated derivatives with the methyl group in (ω-2)- and (ω-3)-position were identified. In the case of (ω-3)-branched 2-carbonitriles, coelution with (ω-2)-branched 1-carbonitriles shorter by one carbon atom was observed as explained for 28-methyltriacontane-1-carbonitrile (28Me-30:1-CN, 461 amu) and 28-methylhentriacontane-2-carbonitrile (28Me-31:2-CN, 475 amu). Both derivatives overlapped in the chromatogram and that can be demonstrated by the calculation of theoretical *RI*s. RI_{theo} for 28Me-30:1-CN was calculated to be 3527 under contribution of 3000 points for the alkyl chain, 454 points for the 1-carbonitrile unit, and 75 points for methyl branch in the (ω-2)-position. Correspondingly, a RI_{theo} of 3535 was predicted for 28Me-31:2-CN by applying the formula of figure 83 and addition of 60 points for the methyl branch in (ω-3)-position. That was confirmed by the experiment because the ion trace m/z = 475 of the 2-carbonitrile occurred subordinately in the ion trace m/z = 461 of the 1-carbonitrile with a slight shift to higher retention times. Both mass spectra were characterized by the ions m/z = 404 and 432, indicating the methyl branch in (ω-2)-position for the 1-methoxy derivative and (ω-3)-position for the 2-methoxy derivative, respectively.

Moreover, branched derivatives in (ω-2)-position occurred in this extract and the mass spectrum of 28-methyltriacontane-2-carbonitrile (28Me-30:2.CN) is depicted in Figure 84.

The ions *m*/*z* = 404 and 432 indicated the (ω-2) methyl group and the calculated *RI* of 3448 was in good agreement with the observed value of 3457.

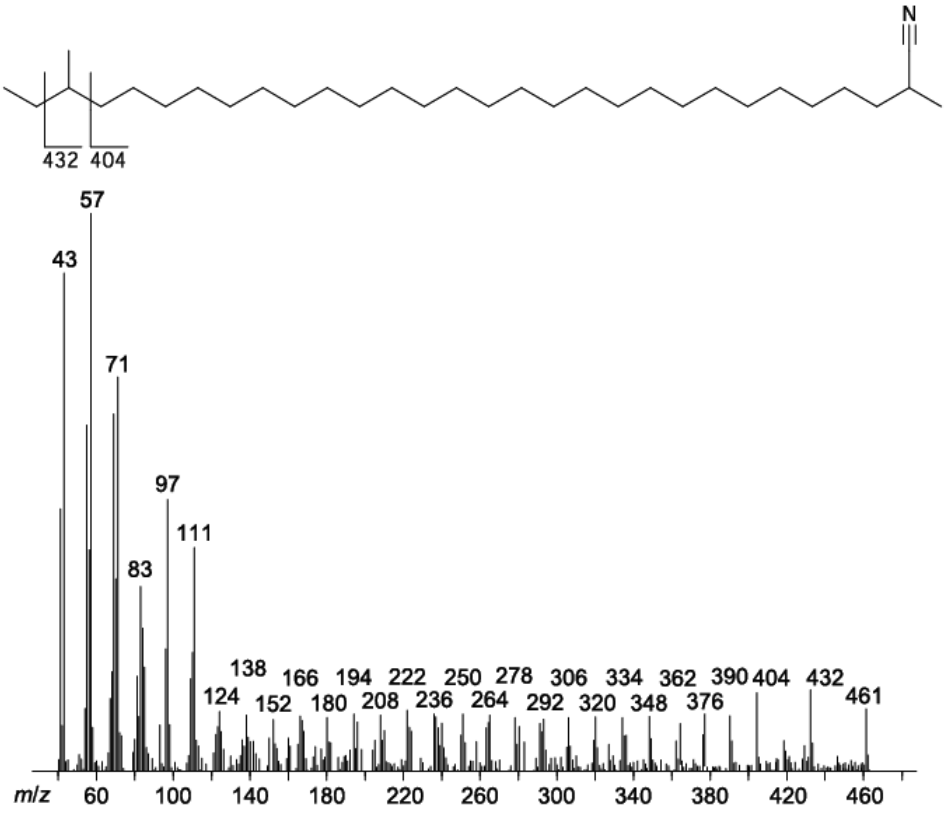

Figure 84: Mass sepctrum of 28-methyltriacontane-2-carbonitrile.

Additionally, a blend of internal branched 2-carbonitriles with 31 carbon atoms was present and these derivatives coeluted. A corresponding mixed mass spectrum is shown in Figure 85. All the derivatives were characterized by the *RI* of 3509. Based on the strong intensity of the ions *m*/*z* = 180, 208, 236, 264, 292, and 320, a blend of 12Me-, 14Me-, 16Me-, 18Me- and 20Me-31:2-CN was elucidated. The experimental *RI* of 3509 was near to the theoretical one of 3510-3515, by addition of 30-35 points for an internal methyl branch.

Derivatives with methyl groups in position 24 and 26 also occurred.

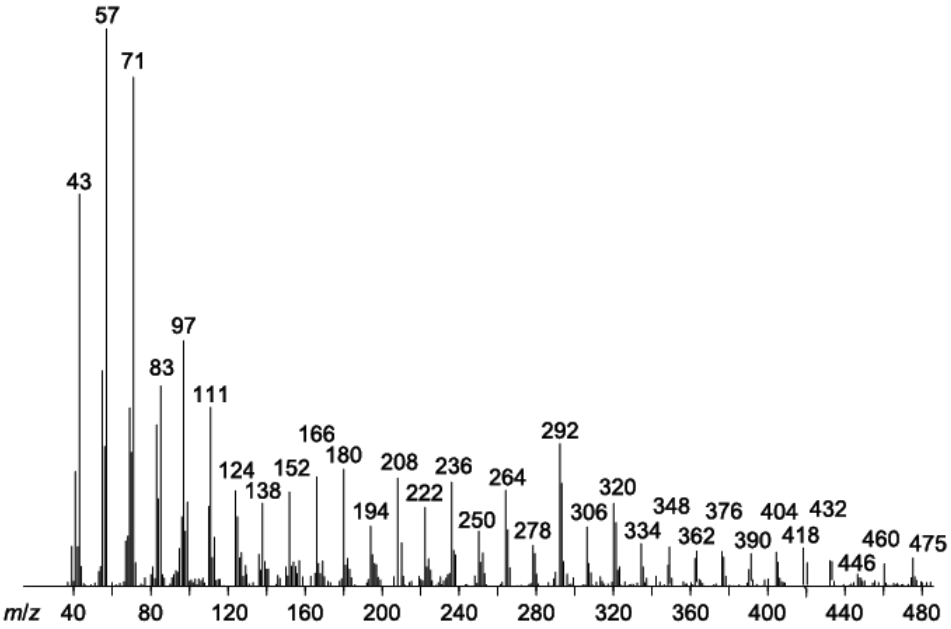

Figure 85: Mass spectrum of a blend of internal branched 2-carbonitriles with a chain length of 31 carbon atoms.

Trimethyl branched derivatives with an internal arrangement of the methyl groups were identified in this extract and structure elucidation is explained for 16,18,22-trimethyltriacontane-2-carbonitrile (16,18,22TriMe-30:2-CN) and its mass spectrum is illustrated in Figure 86.

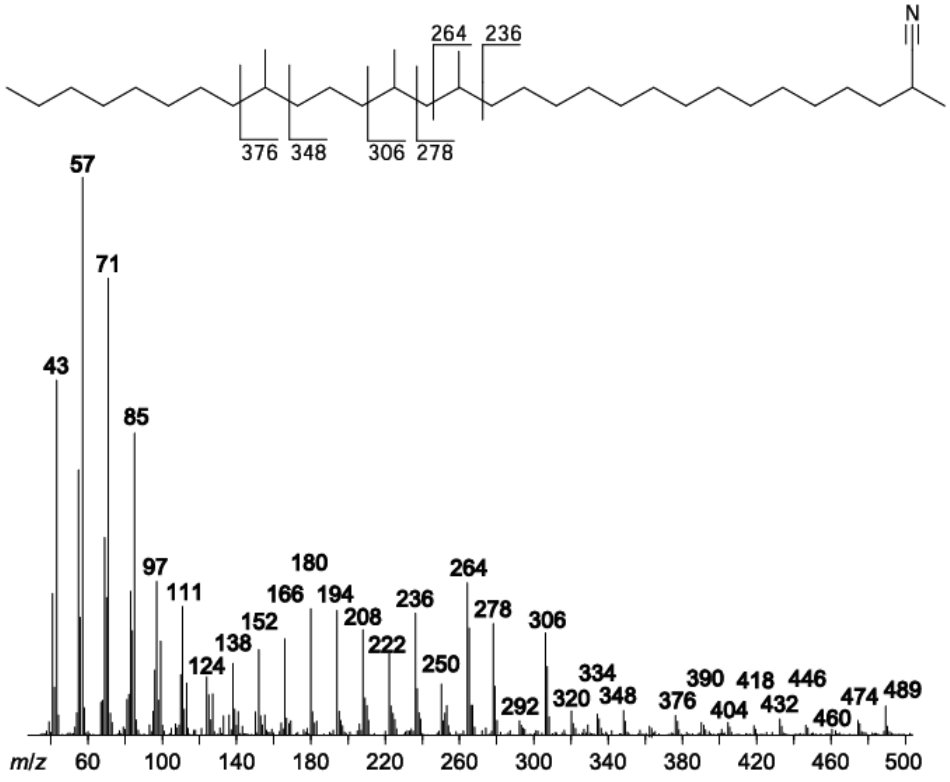

Figure 86: Mass spectrum of 16,18,22-trimethyltriacontane-2-carbonitrile.

The molecular weight of the derivative was 489 amu and the derivative had a *RI* of 3503. The mass spectrum was characterized by the ions *m*/*z* = 236, 264, 278, 306, 348, and 376 indicating the 16,18,22-arrangement of the methyl groups.

Additionally, trimethyl branched derivatives with two internal and one terminal methyl branch were identified. For instance, the mass spectrum of 16,18,26-trimethylnonacosane-2-carbonitrile (16,18,26TriMe-29:2-CN) with a molecular weight of 475 amu was characterized by the ions *m*/*z* = 236, 264, 278, 306, 404, and 432 and thus clearly demonstrating the 16,18,26-arrangement of the methyl groups. This derivative was characterized by a *RI* of 3402 and 3206 for the corresponding methoxyalkane. The calculated *RI* had a value of 3401 for the carbonitrile and a *RI* between 3200-3210 for the methoxyalkane

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3.2.2.3 Composition of the methyl ethers in females of *Diplocephalus permixtus*

Table 13 compiles the composition of the methoxyalkane blend in female *D. permixtus*.

Table 13: Composition of the blend of methoxyalkanes in female *Diplocephalus permixtus*. Abbreviations: MA methoxyalkane; ME methyl ester; CN carbonitrile. Intensity of compounds is given in %.

RI_{MA}	RI_{ME}	RI_{CN}	Compound	Intensity
3112	3219	3333	26Me-28:1-OMe	0.6
3141	-	3339	26Me-29:2-OMe	0.4
3206	-	3402	16,18,26TriMe-29:2-OMe	0.6
3194	3303	3417	26Me-29:1-OMe	1.7
3258	-	3457	28Me-30:2-OMe	0.3
3302	-	3503	16,18,22TriMe-30:2-OMe	1.2
3312	-	3509	12/14/16/18Me-31:2-OMe	1.0
3320	-	3516	20/24Me-31:2-OMe	0.4
3325	-	3524	26Me-31:2-OMe	0.1
3313	3421	3538	28Me-30:1-OMe	1.0
3342	-	3541	28Me-31:2-OMe	1.5
3404	-	3602	18,20,28TriMe-31:2-OMe	0.5
3396	3505	3619	28Me-31:1-OMe	6.8
3396	-	3625	2,16,18,28TetraMe-30:1-OMe	„
3396	3506	3633	2,18DiMe-31:1-OMe	„
3407	3513	3640	2,24DiMe-31:1-OMe	0.5
3415	-	3647	2,26DiMe-31:1-OMe	0.7
3432	3527	3663	2,28DiMe-31:1-OMe	7.6
3440	3537	3671	2,(18/20),26TriMe-31:1-OMe	0.7
3460	3553	3689	2,(20/24),28TriMe-31:1-OMe	0.7
3511	-	3710	20,22,30TriMe-32:2-OMe	2.4

RI_{MA}	RI_{ME}	RI_{CN}	Compound	Intensity
3496	3588	3729	2,18,20,XTetraMe-31:1-OMe	4.5
-	3593	3736	2,20DiMe-32:1-OMe	„
-	3618	3741	2,(22/24)DiMe-32:1-OMe	„
3541	-	3743	30Me-33:2-OMe	0.4
3550	3645	3784	2,30DiMe-32:1-OMe	2.0
3561	3667	3788	18,(20/22),30TriMe-32:1-OMe	5.5
-	-	3799	(20/22/24)Me-33:1-OMe	4.2
3603	3690	-	2,18,20,24TetraMe-32:1-OMe	35.8
3610	3700	-	2,(20/22/24)DiMe-33:1-OMe	„
3635	3729	-	2,30DiMe-33:1-OMe	5.0
3641	3736	-	2,20,28TriMe-33:1-OMe	1.3
3660	3754	-	2,22,30TriMe-33:1-OMe	0.9
3693	3788	-	2,20,22,30TetraMe-33:1-OMe	1.9
3701	3797	-	2,(20/22/24)DiMe-34:1-OMe	1.9
3756	-	-	2,18,22TriMe-34:1-OMe	0.9
3749	-	-	2,32DiMe-34:1-OMe	1.2
-	-	-	2,20,24,XTetraMe-34:1-OMe	2.1
-	-	-	2,(22/24)DiMe-35:1-OMe	3.4

Methyl groups in monomethyl branched 1-methoxyalkanes were found either internally or teminally in the (ω-2)- or (ω-3)-position. The corresponding dimethylated derivatives were often characterized by a methyl group in 2-position and an additional internal methyl group. Three motifs were found in trimethylated derivatives. The methyl groups were either internally or two internally and one near the alkyl end. Furthermore, one methyl group was present in 2-position, another one was attached on one of the internal carbon atoms and the third one was near the alkyl end. The corresponding tetramethylated derivatives had a methyl group at C-2, two internal methyl groups, and the remaining near the alky end. The blend of 1-methoxyalkanes comprised derivatives between 28 and 35 carbon atoms. The most abundant derivative was represented by 1-methoxy-2,18,20,24-tetramethyldotriacontane with 35.8%. In contrast, only mono- and trimethylated 2-methoxyalkanes were observed. Monomethylated 2-methoxyalkanes were characterized by an internal or terminal methyl group. Trimethylated derivatives with three internal methyl groups as well as with two internal and one terminal methyl group were present. The chain length of these derivatives ranged from 29 to 33 carbon atoms and the most abundant compound of this blend was 2-methoxy-20,22,30-trimethyldotriacontane with 2.4%.

3.2.2.4 Biosynthesis of 2-methoxyalkanes

In contrast to 1-methoxyalkanes, the biosynthesis of 2-methoxyalkanes is less obvious. According to the biosynthesis of 2-ketones, a pathway as shown in Figure 87 can be discussed. This pathway is explained for the biosynthesis of hypothetic 2-methoxy-25-methylheptacosane **65**.

Acetyl-CoA **59** is elongated by the addition of a methylmalonate unit to furnish 2-methylbutyric acid **60**. The carbon chain is extended by 12 malonate units to provide **61** or **62** if the carbonyl moiety of the penultimate acetate unit is not immediately removed. The intermediate **61** can be transformed into **62** by *β*-oxidation. A decarboxylation of **62** yields the corresponding 2-ketone **63**. Reduction of **63** to the corresponding alcohol **64** followed by methylation with SAM provides **65**. But, this pathway is in contradiction to the observations made. It forms 2-methoxyalkanes with methyl branches in odd-numbered positions and not in even-numbered as found in *D. permixtus*.

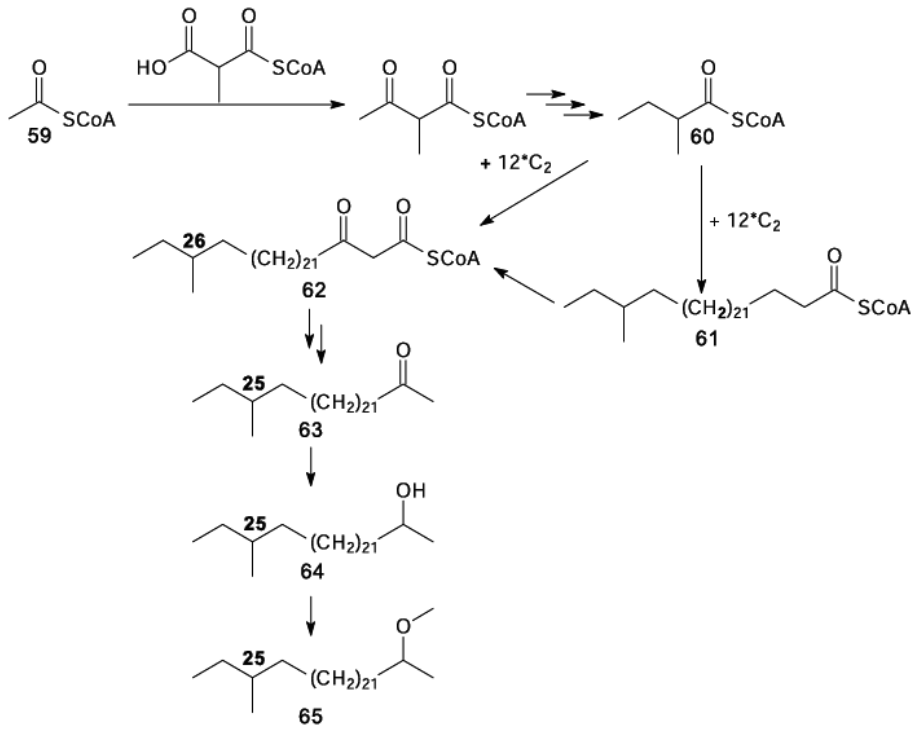

Figure 87: Biosynthesis of 2-methoxyalkanes according to the biosynthesis of 2-ketones.

Thus, an alternative pathway is necessary explaining why the methyl branches occur in even-numbered positions. According to Figure 88, **61** could be reduced to the corresponding alcohol **66** followed by an isomerisation to the 2-alcohol **68**, perhaps via alkene **67** as intermediate. Finally, methylation with SAM furnishes 2-methoxyalkane **69** with the methyl group in an even-numbered position.

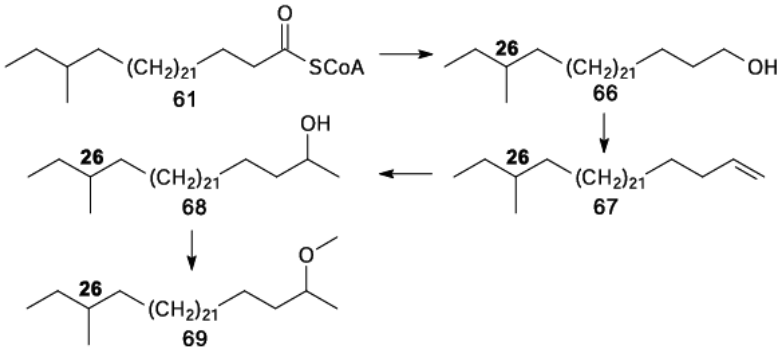

Figure 88: Proposed mechanism for the biosynthesis of 2-methoxy-26-methyloctacosane.

4. Pheromone biosynthesis in the winter moth *Erannis bajaria* and identification of novel compounds in its pheromone gland

4.1 Introduction into the biosynthesis of butterfly pheromones

Butterfly pheromones are subdivided into two groups, called type 1 and type 2 pheromones. Most type 1 pheromones, are characterized by an even-numbered chain length with often 12, 14, or 16 carbon atoms, a polar head group consisting of an alcohol, an acetate, or an aldehyde moiety, and up to three double bonds with (*E*)- or (*Z*)-configuration which are frequently conjugated. Recently, the variety of type 1 structures was reviewed [Ando, Inomata and Yamamoto, 2004]. Characteristic examples of type 1 structures as well as unique structures are illustrated in Figure 89.

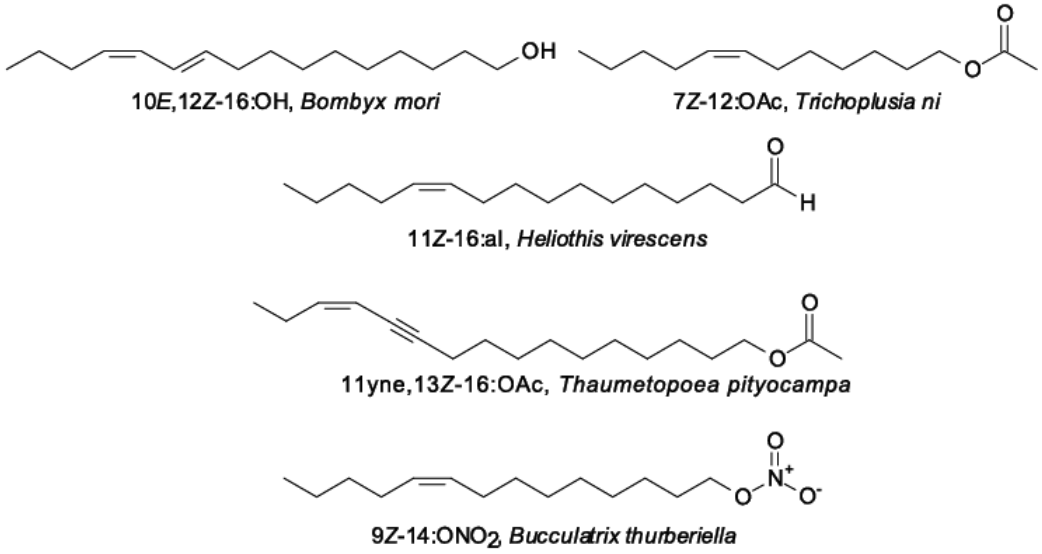

Figure 89: Structures of common type 1 pheromones.

Bombykol, (10*E*,12*Z*)-10,12-hexadecadienol (10*E*,12*Z*-16:OH) was identified as the first butterfly sex pheromone in the silkworm *Bombyx mori* and was the motivation for many researchers in this field of chemical ecology to perform studies about pheromone systems in butterflies [Butenandt *et al*., 1959]. Later, also (10*E*,12*Z*)-10,12-hexadecadienal (10*E*,12*Z*-16:al) was elucidated as constituent of the pheromone blend of *B. mori* [Kaissling *et al.*, 1978]. The pheromone blend of the cabbage looper, *Trichoplusia ni*, comprises (*Z*)-7-dodecenyl acetate (7*Z*-12:OAc), a widespread semiochemical in over 100 butterfly species and further investigations proved that (*Z*)-7-dodecenol (7*Z*-12:OH) and dodecyl acetate (12:OAc) are also part of this pheromone blend. [Haynes and Hunt, 1990; Dunkelbaum and Mazor, 1993]. The pheromone system of the tobacco budworm moth, *Heliothis virescens* consists of (*Z*)-11-hexadecenal (11*Z*-16:al), also very common

in butterflies, and (*Z*)-9-tetradecenal (9*Z*-14:al) [Roelofs *et al.*, 1974]. These examples demonstrate that species specific pheromone blends are created by blends bearing the same functional group with different chain length, different functional groups with equal chain length, or different number of unsaturations.

Alcohols and aldehydes are derived by reduction of the corresponding fatty acids [Morse and Meighen, 1987]. But aldehydes are also formed by oxidation of the corresponding alcohols as shown in the cotton wool pest *Heliothis zea* and the tobacco pest *Manduca sexta* [Teal and Tumlinson, 1988; Fang *et al.*, 1995]. Aldehydes are not inevitable intermediates in the formation of alcohols. For example, bombykol is formed by reduction of the corresponding acid without an aldehyde as intermediate [Moto *et al*., 2003]. Acetates are formed by acetyl transferases which are often not substrate specific [Ando, Inomata and Yamamoto, 2004]. But sometimes, specifity of acetyl transferases can affect the composition of a pheromone blend [Jurenka and Roelofs, 1989; Liu *et al*., 2002]. The initial blend of *Argyrotaenia velutinana* contains a mixture of 11*Z*-14:CoA/11*E*-14:CoA in a ratio of 6:1 but the final mixture consists of a 92:8-blend of 11*Z*-14:OAc/11*E*-14:OAc caused by a higher affinity of the acetyl transferase for the corresponding (*Z*)-alcohol. Other enzymes are also capable to influence the final pheromone blend. The pheromone blend of the European corn borer *Ostrinia nubilalis* comprises two strains, a (*E*)- and a (*Z*)-strain, and the latter one is characterized by a *Z*/*E*-mixture of 11-tetradecenyl acetate at least in a ratio of 97:3. The active mixture of the (*E*)-strain comprise a *Z*/*E*-mixture in a ratio of 1:99. In both cases, the corresponding acids are formed in a ratio of 30:70 and (*E*)- and (*Z*)-specific reductases provide finally the blends of the (*Z*)- and (*E*)-strain [Wolf and Roelofs, 1987; Zhu *et al.*, 1996; Roelofs *et al.*, 2002].

Rarely, compounds with a triple bond or a nitric acid group occur. Hexadeca-11-yne-13-enyl acetate (11yne,13*Z*-16:OAc) was identified in *Thaumetopoea pityocampa* and the triple bond is generated by a 11-acetylenase [Guerrero *et al.*, 1981; Arsequell *et al.*, 1990]. A mixture of (*Z*)-9-tetradecenyl nitrate and (*Z*)-8-tridecenyl nitrate was described as pheromone blend in *Bucculatrix thurberiella* [Hall *et al.*, 1992].

Type 1 pheromones are produced in the PG which developed from the modification of epidermal cells. This gland is located between the 8th and 9th abdominal segment in moths and all necessary enzymes are located in this gland [Ma and Ramaswamy, 2003]. The biosynthesis of these pheromones is well studied and the catabolic modification of often occurring fatty acids with 16 and 18 carbon atoms is an early step in the biosynthesis. Then, chain shortening enzymes, desaturases and functional group modifying enzymes adjust a species specific pheromone blend.

The chain length is regulated by the number of β-oxidation cycles shortening fatty acids precursors as palmitic acid (16:COOH) and stearic acid (18:COOH). These enzymes are present in the peroxisomes comparable to mitochondria concerning the ability to perform β-oxidation. Key enzymes for chain-shortening are the acyl-CoA-oxidase and the 3-oxoacyl-CoA-thiolase. The first one is a multifunctional enzyme comprising an enoyl-CoA-synthase, enoyl-CoA-hydratase and a 3-hydroxyacyl-CoA-dehydrogenase [Bosch *et al.*, 1992; Hashimoto, 1996]. Desaturases are able to incorporate double bonds in many different positions and thus the variety of butterfly pheromones is enhanced by species specific desaturases [Ando, Inomata and Yamamoto, 2004]. Recently, a study demonstrated a method causing different pheromone blends in two closely related species, the European corn borer *O. nubilalis* and the Asian corn borer *O. furnacalis* [Roelofs *et al.*, 2002]. The pheromone blend of the Asian corn borer consists of a *E*/*Z*-mixture of 12-tetradecenyl acetate [Cheng *et al.*, 1981] whereas the European corn borer uses a *E*/*Z*-mixture of 11-tetradecenyl acetate [Klun *et al*., 1973]. Investigations indicated clearly that both genomes contain the gene sequence for both desaturases but that one transcript is nonfunctional in the genome of these species.

In general, female butterflies rather use pheromone blends in a well defined ratio of compounds than a single compound and an accurate cooperation of key enzymes is necessary for their formation [Jurenka, 2004].

Type II pheromones are polyunsaturated hydrocarbons and corresponding epoxides resulting from the oxidation of the double bonds [Millar, 2000]. Characteristic examples are depicted in Figure 90. These pheromones are predominantly used by a few macrolepidopteran families including the geometridae, acrtiidae, some subfamilies of noctuidae and lymantriidae. The biosynthesis of these pheromones is derived from linoleic acid or α-linolenoic acid and results in odd numbered hydrocarbons, as for example the widespread (3*Z*,6*Z*,9*Z*)-3,6,9-tricosatriene (3Z,6Z,9Z-23:H), with an (6*Z*,9*Z*)- or (3*Z*,6*Z*,9*Z*)-arrangement of the double bonds [Rule and Roelofs, 1989; Millar, 2000]. These compounds are formed by chain extension of linoleic and linolenoic acid with acetate via malonate to provide acids with an appropriate chain length, followed by reduction to the corresponding aldehydes, and final decarbonylation or decarboxylation to furnish the odd-numbered carbon skeleton [Cheesbrough and Kolattukudy, 1984; Yoder *et al.*, 1992; Reed *et al.*, 1994]. The decarbonylation proceeds without need of oxygen or cofactors whereas the decarboxylation requires oxygen, $NADP^+$ and a cytochrome P 450 as catalyst under delivery of carbon dioxide [Reed *et al.*, 1995]. Pheromone blends bearing even-numbered hydrocarbons or the corresponding epoxides are less frequent and only a few examples were mentioned in two closely related species of geometrid moths. The epoxydiene, (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxyoctadeca-3,6-diene

(3*Z*,6*Z*,9*S*,10*R*-epo-18:H) was described as sex attractant in field tests with *Hemerophila atrilineata* but a racemic mixture showed similar activity compared to the (9*S*,10*R*)-enantiomer [Pu *et al.*, 1999]. The triene, (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene (3*Z*,6*Z*,9*Z*-18:H) is essential for the maximum activity of the pheromone blend of the winter moth *Erannis bajaria*, consisting of a 1:1-mixture of 3*Z*,6*Z*,9*Z*-18:H and 3*Z*,6*Z*,9*Z*-19:H [Plaß, 1999]. Corresponding even-numbered acids were proposed as precursor for these compounds [Millar, 2000].

Hydrocarbons are produced in oenocytes, located in the epidermis, independent of their local requirement. The hydrocarbons are transported through the hemolymph to their different domains by a special transport protein, called lipophorin [Schal *et al.*, 1998a, 1998b]. There, the hydrocarbons are recognized by special receptors.

Epoxidations are performed by monooxygenases. Investigations demonstrated that this step proceeds in the PG whereas all other steps occur outside of the PG [Miyamoto *et al.*, 1999; Wei *et al.*, 2004]. Their conclusions based on the incorporation of deuterated hydrocarbons into the final epoxides after topical application or injection of labeled precursors to the PG. Furthermore, these enzymes work regiospecific. On the other hand, these enzymes are not able to distinguish between different chain lengths and thus, every offered hydrocarbon is epoxidized independent of its chain length. Regiospecific epoxidations and the resulting chiral compounds increase the diversity of type II compounds. Chiral expoxides have a considerable impact of the attractivity of a pheromone blend for conspecific males [Millar *et al.*, 1990]. Both enantiomers of (6*Z*,9*Z*)-cis-3,4-epoxyheptadeca-6,9-diene (6*Z*,9*Z*-6,9-cis-3,4-epoxy-17:H) act synergetically in male trapping and this racemic mixture is more attractive than the single enantiomers in the geometrid moths *Semiothisa signaria dispuncta* and *Epelis truncataria*. The diepoxide (3*Z*)-6,7,9,10-diepoxyhenicos-3-ene (3*Z*-*cis*-6,7-*cis*-9,10-diepo-21:H) was described in *Leucoma salicis* (lymantriidae) as the first semiochemical bearing a diepoxide moiety [Gries *et al*., 1997].

Furthermore, additional double bonds increase the diversity of type II pheromones as reported in the winter moth *Operophtera brumata*. Its pheromone is (1,3*Z*,6*Z*,9*Z*)-1,3,6,9-nonadecatetraene (1,3*Z*,6*Z*,9*Z*-19:H) [Roelofs *et al.*, 1982]. Putatively, the additional double bond is introduced before the final decarbonylation or decarboxylation occurs because (11*Z*,14*Z*,17*Z*)-11,14,17,19-eicosatetraenoic acid (11*Z*,14*Z*,17*Z*,19-20:Ac) was detected in extracts of this moth [in Millar, 2000; unpublished data of Zhao and Löftstedt].

Unusual pheromones derived by linoleic or linolenoic acid appear in several arctiid moths. Their pheromone blend comprises (9*Z*,12*Z*)-9,12-octadecadienal (9*Z*,12*Z*-18:al) and

(9Z,12*Z*,15*Z*)-9,12,15-octadecatrienal (9Z,12*Z*,15*Z*)-18:al and these compounds are provided by reductive modifications of the corresponding acids [Hill and Roelofs, 1981; Rule and Roelofs, 1989]. Females of the douglas-fir tussock moth *Orgyia pseudotsugata* produce (*Z*)-6-henicosen-11-one (6*Z*-21:11one); another example for an unusual butterfly sex pheromone [Smith *et al.*, 1975]. These two examplaric compounds contain characteristics of type I and type II pheromones.

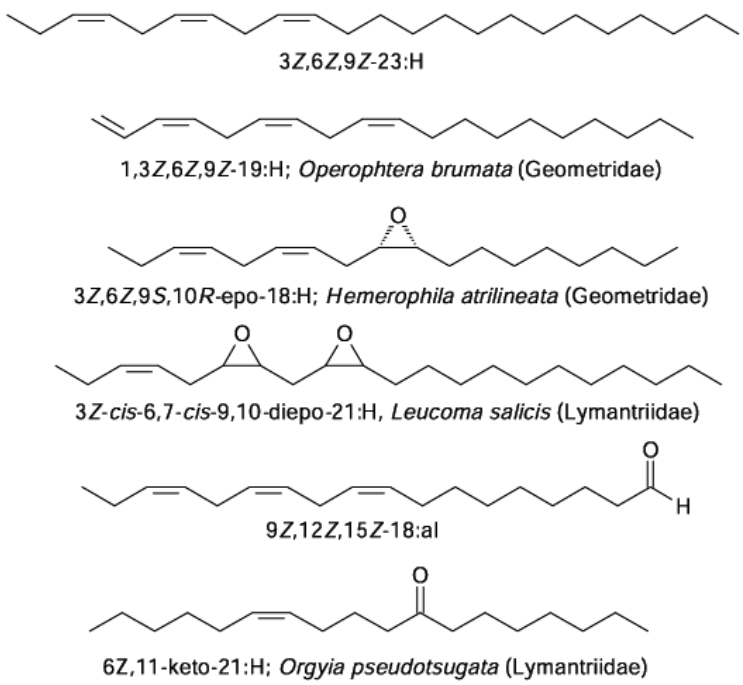

Figure 90: Overview of common type II pheromones and compounds containing elements of type I and type II pheromones.

Species specific pheromone blends containing type II compounds are also composed of compounds of mixed chain length, different numbers of double bonds, and different functional groups.

Investigations about the regulation of biosynthetic pathways in butterflies are increasing in importance [Jurenka, 2004]. Regulating mechanisms are essential to ensure the timing between pheromone biosynthesis and calling period. The pheromone biosynthesis activating neuropeptide (PBAN) plays a central role in this context. This peptide was identified in *H. zea* in the subesophageal ganglion and the purified peptide was able to induce pheromone biosynthesis in head-ligated females during the photophase [Raina *et al.*, 1987]. This peptide comprises a sequence of 33 amino acids (AAs). Furthermore, this peptide triggered pheromone biosynthesis in six other moth species indicating that this principle of regulation is widespread [Raina *et al.*, 1989]. Subsequent investigations showed a core sequence with an amidated C-terminal FSPRL

(Phe-Ser-Pro-Arg-Leu-NH_2) to be essential for a minimum of activity [Raina and Kempe, 1990].

The PBAN receptor in the membrane of the PG of *H. zea*, a producer of type I pheromones, was identified and a mechanism for the mode of action of PBAN was proposed [Choi *et al.*, 2003]. Coupling of PBAN to the receptor enhances cytosolic Ca^{2+}-concentration by transport of extracellular Ca^{2+} [Jurenka *et al.*, 1991]. These ions bind to the peptide calmodulin which acts in different ways on enzymes affecting pheromone biosynthesis [Iwanaga *et al.*, 1998]. In heliothine moths like *H. zea*, that modulates an adenylate cyclase which converts adenosin triphosphate (ATP) to cyclic adenosyl monophosphate (cAMP) followed by activation of a phosphatase influencing an important enzyme in the biosynthetic pathway. This cascade results in an increased pheromone production [Jurenka, 1996].

Another mode of action was decribed in butterflies using type 2 pheromones. In the geometrid species *H. artrilineata* and *Ascotis selenaria cretacea*, PBAN increases the concentration of precursors for the epoxyderivatives in the PG [Wei *et al.*, 2004]. In manipulation experiments with decapitated females, the epoxides disappeared after 36h but they were recovered after application of deuterated precursors to the PG indicating that PBAN had no impact on the oxygenase enzymes. Decapitated females lacking epoxides restarted formation of them after injection of *Bombyx mori* PBAN and deuterated precursors into the abdomen demonstrating an enhanced precursor transport to the PG mediated by PBAN.

4.2 Biosynthesis of (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene in *Erannis bajaria* (Lepidoptera:Geometridae)

4.2.1 Considerations about its biosynthesis

The biosynthesis of type II pheromones with an odd number of carbon atoms is well-known in contrast to that of the even-numbered ones.

Direct reduction of the acid moiety of polyunsaturated even-numbered fatty acids **70**, perhaps with corresponding aldehydes **71**, alcohols **72**, and alkenes **73** as intermediates, was proposed and the corresponding pathway is shown in Figure 91 for 3*Z*,6*Z*,9*Z*-18:H **74** [Millar, 2000]. First, **72** is formed by two reduction steps follwed by elimination of water to provide **73**. Finally, **73** is hydrogenated in the presence of nicotinamide adenine dinucleotide (phosphate) NAD(P)H or flavin adenine dinucleotide ($FADH_2$) to furnish **74**. This assumption based on the identification of even-numbered aldehydes in several species of arctiidae derived from corresponding acids [Rule and Roelofs, 1989]. Recently, biosynthesis of *n*-alkanes via corresponding 1-alcohols was

demonstrated in a bacterium [Park, 2005].

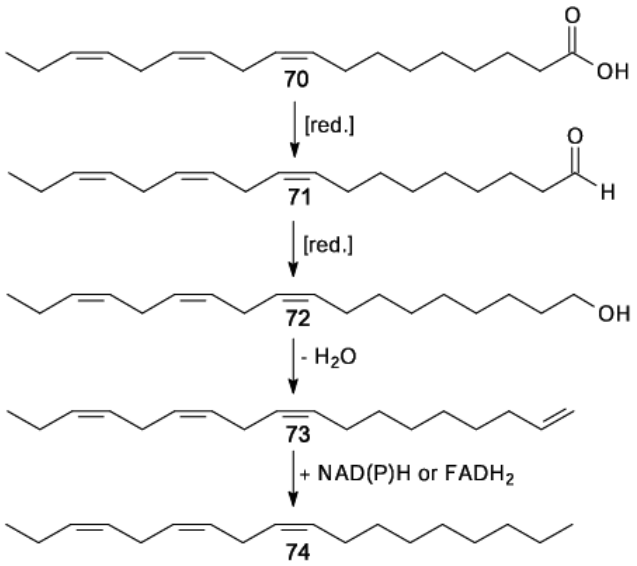

Figure 91: Biosynthesis of (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene [Millar, 2000].

In contrast, another biosynthetic pathway, based on our idea, is illustrated in Figure 92 for **74**. First, **70** is elongated by acetate (malonate) to form (11*Z*,14*Z*,17*Z*)-11,14,17-eicosatrienoic acid **75**, followed by α-oxidation to form (10*Z*,13*Z*,16*Z*)-10,13,16-nondecatrienoic acid **76**. Finally, **76** is converted into **74** by reduction to (10*Z*,13*Z*,16*Z*)-10,13,16-nondecatrienal **77** and subsequent decarbonylation or decarboxylation.

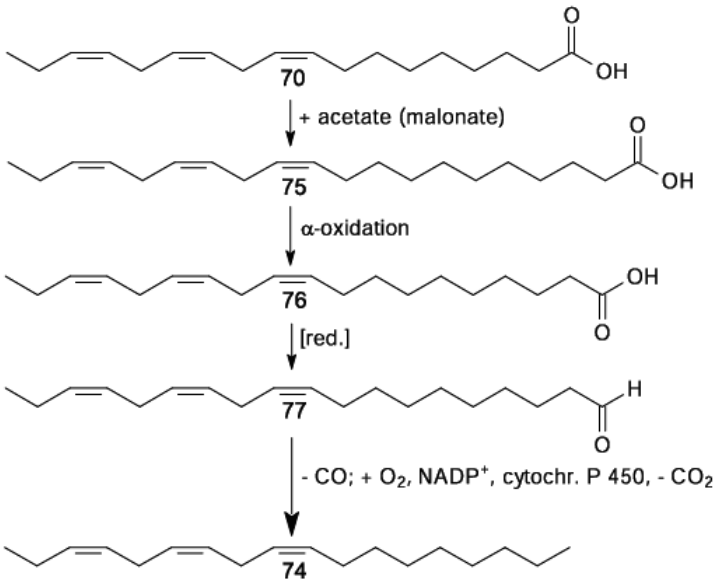

Figure 92: Alternative biosynthetic pathway for (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene.

The pathway of Millar needs two independent steps for the formation of the 1:1-blend of 3*Z*,6*Z*,9*Z*-18:H and 3*Z*,6*Z*,9*Z*-19:H in *E. Bajaria*. In contrast, pathways for the biosynthesis of both pheromones overlap partly in our considerations because chain elongation provides initially **75**. After that, this compound serves as direct precursor for the biosynthesis of both pheromones.

Both hypotheses were investigated by applying deuterium labeled precursors to *E. bajaria* before the daily pheromone production started followed by GC-MS analysis of the resulting pheromone blend. "Feeding experiments" were performed by Dr G. Szöcs (Budapest).

4.2.2 Syntheses of labeled pheromone precursors

The deuterium labeled precursors (9*Z*,12*Z*,15*Z*)-1,1,2,2-tetradeuterooctadeca-9,12,15-trien-1-ol **93**, (9*Z*,12*Z*,15*Z*)-9,10,12,13,15,16-hexadeuterooctadeca-9,12,15-trienoic acid **90**, (10*Z*,13*Z*,16*Z*)-2,2,3,3-tetradeuterononadeca-10,13,16-trienoic acid **98**, and (11*Z*,14*Z*,17*Z*)-3,3,4,4-tetradeuteroeicosa-11,14,17-trienoic acid **102** were synthesized by two strategies.

An acetylenic approach was used to synthesize **90** (Figure 93).

Compound **79** was obtained in 71% yield by protection of propargylic alcohol **78** with 3,4-dihydropyrane. A Cu^{+}-mediated coupling reaction between **79** and 1-bromo-pent-3-yne **80** furnished the THP-protected diyne **81** in 83% yield [Lapitskaya *et al.*, 1993]. The yield was increased by periodic addition of **80**. The first half of the amount was added at the beginning of the reaction and the second part after a reaction time of 8h. Bromide **82** was obtained by treating **81** with bromine and triphenyl phospane in 84% yield [Sonnet, 1976]. Diol **83** was monobrominated with 48% aqueous hydrobromic acid to compound **84** in only 19% yield. The reaction time was short to avoid excess formation of the dibrominated side product. Unconsumed educt was recovered by separation of the products from the aqueous crude mixture. THP-ether **85** was formed by protection of **84** with 3,4-dihydropyrane in 94% yield. Chain elongation of **85** with lithium acetylid provided **86** in 77% yield [Jayasuria *et al.*, 1990]. Finally, **82** and **86** were coupled to triyne **87** as described for **81**. A turnover of 60% was observed by GC but **87** was isolated in only 35% yield caused by difficult separation from the educts. Conversion of **87** into the corresponding deuterated triene **88** in a titanium(II)-based *Z*-reduction proceeded in low yield only [Hungerford and Kitching, 1998]. Furthermore, the compound was impure and contained mainly only 4 on the double bonds randomly distributed deuterium atoms. Deprotection of crude **88** furnished alcohol **89** and final oxidation with PCC formed acid **90**.

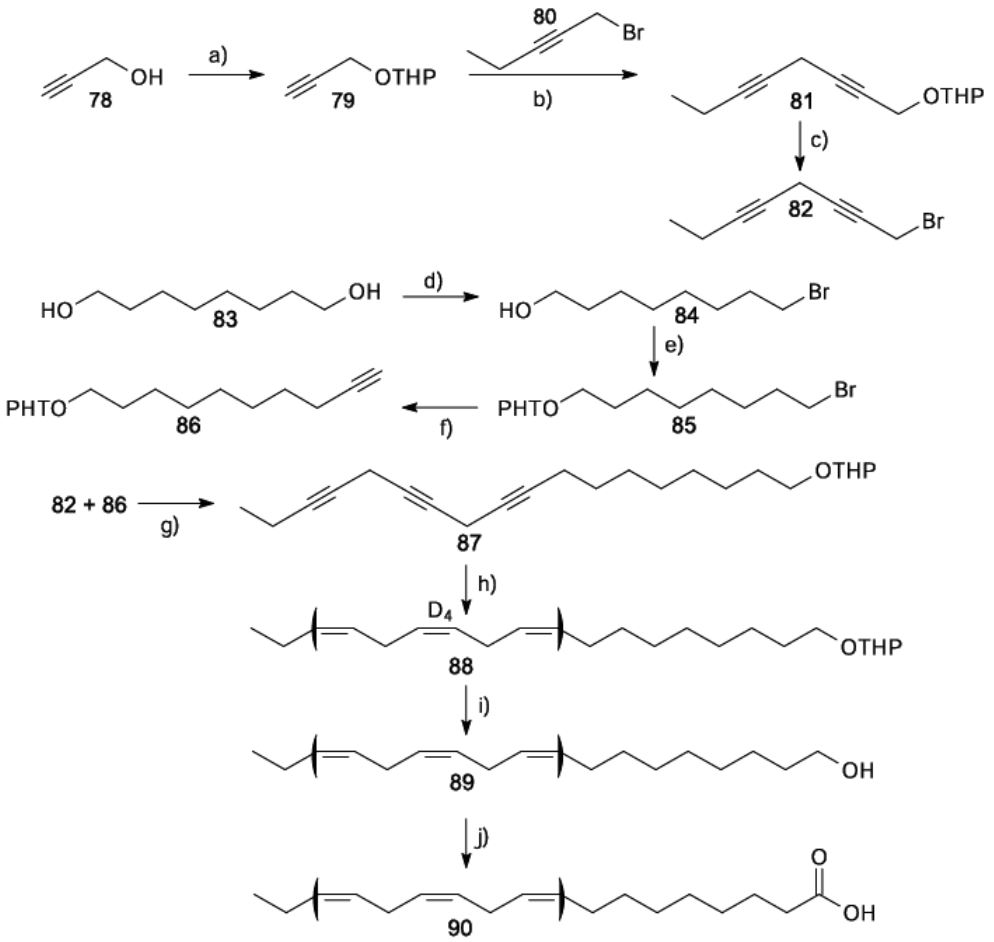

Figure 93: Synthesis of **90**. a) Et_2O, *p*-TsOH, DHP, 24h rt, 71%; b) DMF, K_2CO_3, CuI, NaI, 48h rt, 83%; c) DCM, PPh_3, Br_2, 16h rt, 82%; d) 48% aqu. HBr, 8h reflux, 19%; e) Et_2O, *p*-TsOH, DHP, 24h rt, 94%; f) DMSO, Li-acetylid, 2h 0°C, 16h rt, 77%; g) DMF, K_2CO_3, CuI, NaI, 48h rt, 35%; h) *i*PrMgBr:Ti(O*i*Pr)$_4$ 20:8, -78°C, 2h -30°C, -78°C + D_2O, i) MeOH, *p*-TsOH, 16h rt; j) DMF, PDC.

The precursors **93**, **98**, and **102** with the deuterium labeling near the polar head moieties were prepared according to Figure 94 and 95.

Commercial available methyl α-linolenoate **91** was treated with sodium methoxide in refluxing [D_1]-methanol and furnished the dideuterated compound **92** in 95% yield. Reduction of **92** with lithium aluminium deuteride (LAD) provided the tetradeuterated alcohol **93** in 89% yield. The corresponding iodide **94** was obtained under Mitsunobu conditions in 69% yield [Manna *et al.*, 1985]. Alkyl cyanide **95** was prepared in 89% yield by nucleophilic substitution with tetraethylammonium cyanide followed by reduction with DIBAL in 50% yield to provide aldehyde **96**. Direct oxidation of **96** to acid **98** failed because the triene moiety was sensitive to oxidation

using Tollens conditions with silver (I) oxide. Alternatively, **96** was reduced to alcohol **97** with LAH in 92% yield and final oxidation with PDC in DMF furnished **98** in 30% yield. Furthermore, **98** was converted into alkyl cyanide **99** in 61% yield by alkylation with deprotonated acetonitrile [Taber and Kong, 1997]. Final steps for the synthesis of acid **102** were performed as described in the synthesis of acid **98**.

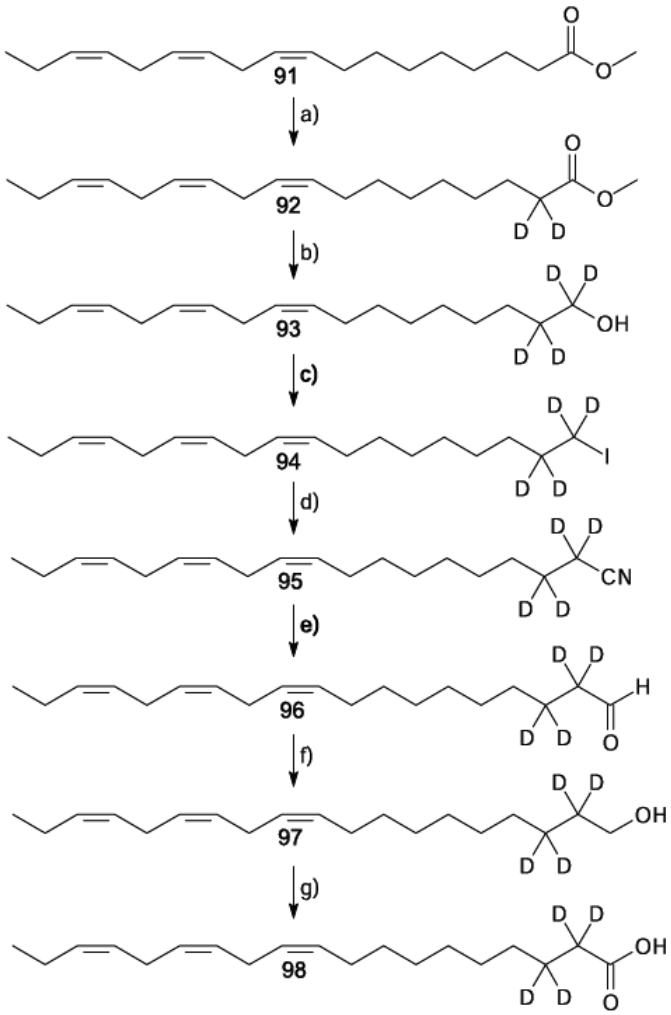

Figure 94: Synthesis of **93** and **98**. a) Na, [D_1]-MeOH, 4h reflux, 95%; b) Et_2O, LAD, 3h reflux, 89%; c) THF, PPh_3, LiI, NaI, DEAD, 0.5h 0°C, 18h rt, 69%; d) DMSO, NEt_4CN, 2h rt, 89%; e) toluene, DIBAL-H, 2h rt, 50%; f) Et_2O, LAH, 3h, 84%; g) DMF, PDC, 48h rt, 30%.

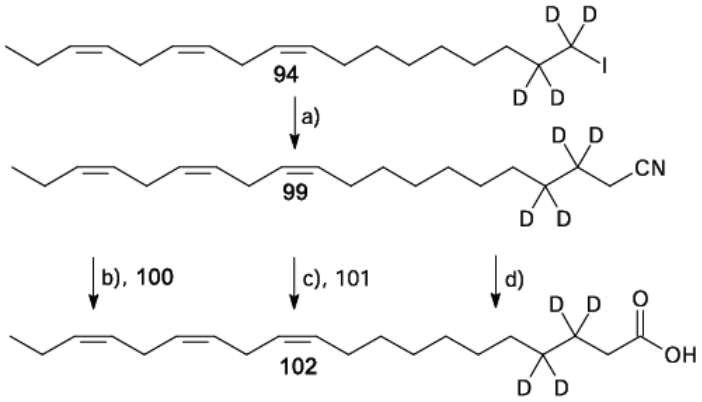

Figure 95: Synthesis of **102**. a) THF, *n*-BuLi, CH_3CN, 2h -78°C, 16h rt, 61%; b) 54%; d) 85%; e) 25%.

The different location of the deuterium label affects the mass spectrum of 3*Z*,6*Z*,9*Z*-18:H (Figure 96).

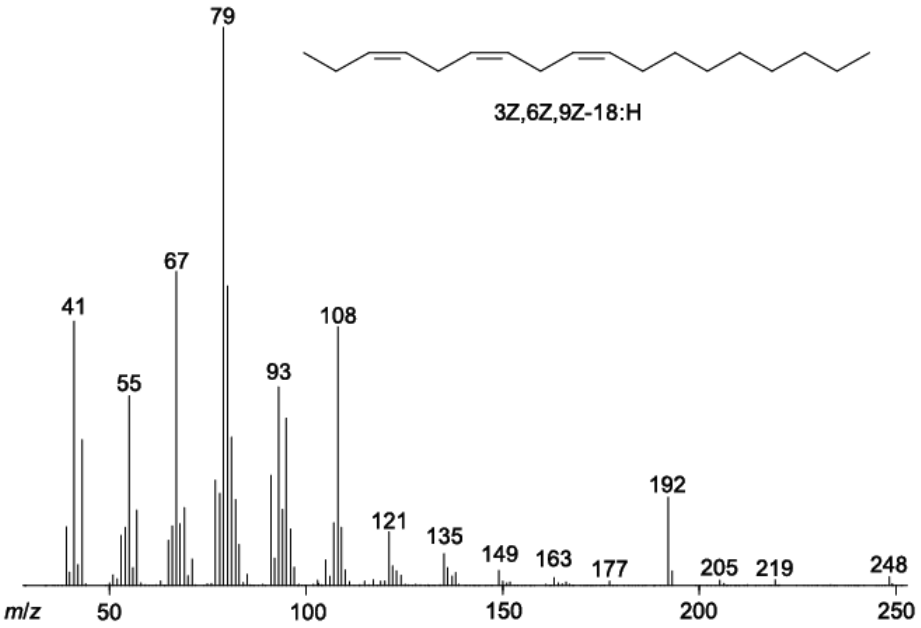

Figure 96: Mass spectrum of (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene **74**.

The mass spectrum is characterized by the base ion *m*/*z* = 79 and in the lower mass region, the ions *m*/*z* = 93 and 108 are important. The first two ions are formed by undefined fragmentation pathways and are directly surrounded by other ions with distinct intensities. Thus, the number of deuterium atoms in these fragments of labeled **77** is unpredictable. On the other hand, the ion *m*/*z* = 108 is useful to assess the deuterium label beccause the fragmentation pathway for this ion is known [Millar, 2000]. A cleavage between C8 and C9 with an hydrogen transfer to the shorter

fragment is responsible for its formation and that is directly affected by a deuterium label in the double bond system as expected for the "feeding" of **90**. Precursors labeled close to the saturated end have no impact on the fragment ions in the lower mass region because these ions are dominated by fragmentations in the double bond system or near them. The ion *m/z* = 192 (M^+ - C_4H_8) is influenced because it is formed by a cleavage between C-4 and C-5 followed by hydrogen transfer resulting in the loss of butene. Thus, a deuterim label close to the end of the alkyl chain evokes a shift for this ion as expected when **93**, **98**, and **102** are fed [Becker *et al.*, 1983].

4.2.3 Results of labeling experiments and discussion

Because of the low pheromone titer, samples of about 15 female equivalents were pooled for analyses. After topical application of **98**, [D_4]-17,17,18,18-3*Z*,6*Z*,9*Z*-18:H was detected in the extracts, along with the unlabeled compound. Application of **102** also yielded the labeled triene **74**, but in lesser amounts. In the latter case, all four ^{2}H-atoms of the labeled acid were incorporated into 3*Z*,6*Z*,9*Z*-19:H. Incorporation of the deuterium label into the polyenes was investigated by GC-MS in multiple ion monitoring mode (MIM). In the case of [D_4]-17,17,18,18-3*Z*,6*Z*,9*Z*-18:H, the molecular ion (M^+) of the unlabeled compound (*m*/z = 248) and its diagnostic fragment M^+-56 (*m/z* = 192) shifted by 4 amu to *m/z* 252 and *m/z* 196, respectively. Table 14 and Figure 97 show the obtained results for the application of **98** and **102** when MIM with the ions *m/z* = 192, 196, 248, and 252 was performed. Mean incorporation rates of 5.7 % in the case of **98** and 1.9 % in case of **102** were calculated from the ion intensities $I_{196}/(I_{192} + I_{196})$ and $I_{252}/(I_{252} + I_{248})$ based on the time interval given in Figure 97.

These results clearly demonstrate that unlabeled **98** and **102** are biosynthetic precursors of 3*Z*,6*Z*,9*Z*-18:H. Furthermore, when topically applied to the gland, **93** did not provide labeled 3*Z*,6*Z*,9*Z*-18:H, which rules out the possibility that linolenoic acid is directly reduced via the alcohol to 3*Z*,6*Z*,9*Z*-18:H, as proposed by Millar (2000). Besides, incorporation of **90** was also not observed in the full scan mass spectrum of 3*Z*,6*Z*,9*Z*-18:H. Perhaps, the incorporation rate was below the detection limit. In general, incorporation rates of early compounds in a biosynthetic pathway are lower in contrast to the later intermediates.

Thus, biosynthesis of 3*Z*,6*Z*,9*Z*-18:H proceeds according to Figure 98. Linolenoic acid **103** , taken up from the diet and most likely conjugated to CoA, is chain-elongated in the conventional sequence with malonate to yield 11*Z*,14*Z*,17*Z*-20:CoA **104**. Then, α-oxidation results in loss of one carbon to give 10*Z*,13*Z*,16*Z*-19:CoA **105** followed by reduction to aldehyde **106**. Finally, **106** is converted into 3*Z*,6*Z*,9*Z*-18:H **74**. In contrast, removal of C-1 from **104** following the conventional

process furnishes 3*Z*,6*Z*,9*Z*-19:H **107**.

The α-oxidation of unbranched fatty acids in animals has not been reported before, although α-oxidation of linolenic acid has been shown to occur in humans (Matsunaga *et al.*, 1997). This mechanism must now be considered to be involved in lipid biosynthesis in other species as well.

Table 14: Relative intensities of ions found in GC-MS multiple ion monitoring analysis at the retention time of 3*Z*,6*Z*,9*Z*-18:H. The earlier elution of the deuterated isotopomer is accounted for by summing up the intensities of the ions from the start until the end of both peaks. I_y: relative intensity of the ion of *m/z* y (%).

results with **98**

m/z	Intensity	Sum 192 + 196	Sum 248 + 252	Incorporation rate in % *m/z* 192, 196	Incorporation rate in % *m/z* 248, 252	Average
192	100.0	106.5	105.6	6.1	5.3	5.8
196	6.5					
248	100.0					
252	5.6					

results with **102**

m/z	Intensity	Sum 192 + 196	Sum 248 + 252	Incorporation rate in % *m/z* 192, 196	Incorporation rate in % *m/z* 248, 252	Average
192	100.0	101.1	102.7	1.1	2.6	1.9
196	1.1					
248	100.0					
252	2.7					

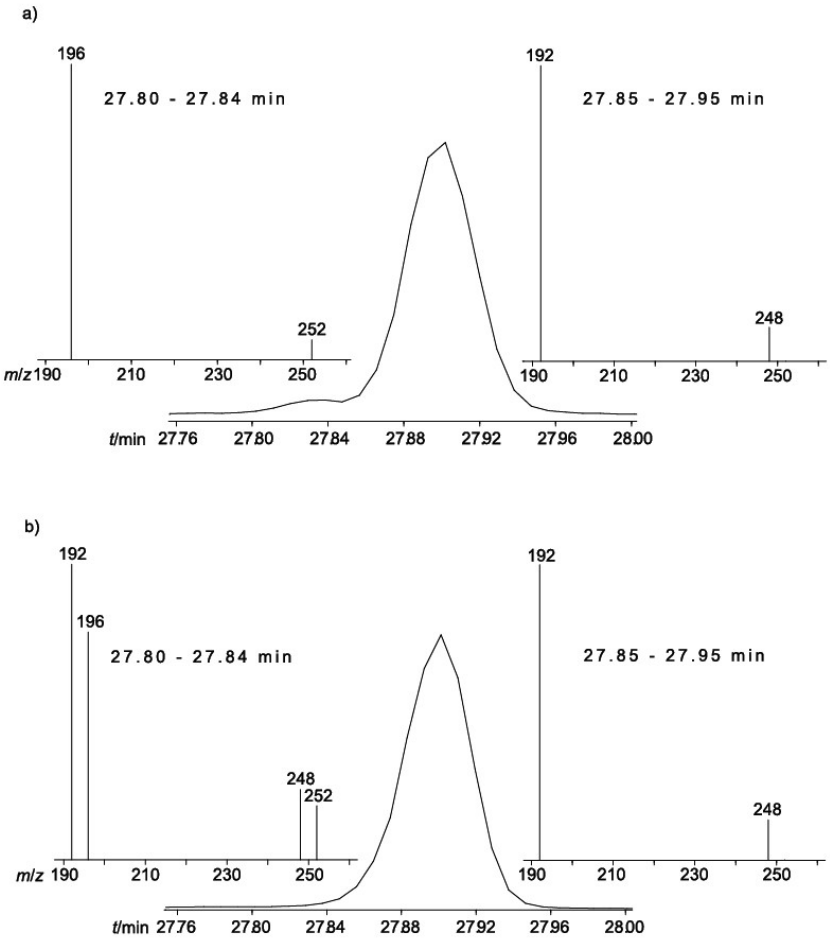

Figure 97: Parts of total ion chromatograms and MIM-spectra with **98** (a) and **102** (b).

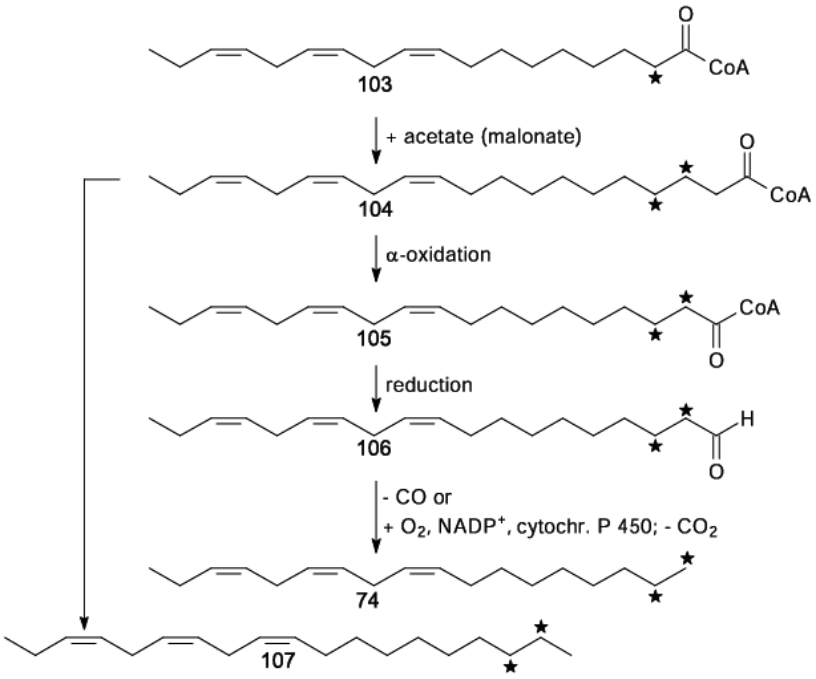

Figure 98: Proposed biosynthetic pathway for the formation of 3*Z*,6*Z*,9*Z*-18:H; * = deuterium label.

4.3 Identification of novel compounds in the pheromone gland of *Erannis bajaria*

4.3.1 Comparison of a pheromone gland extract of *Erannis bajaria* in the year 1996 and 2006

Novel GC-MS-analysis of PG extracts of calling *E. bajaria* revealed the presence of additional components, which were absent in earlier investigations (Figure 99).

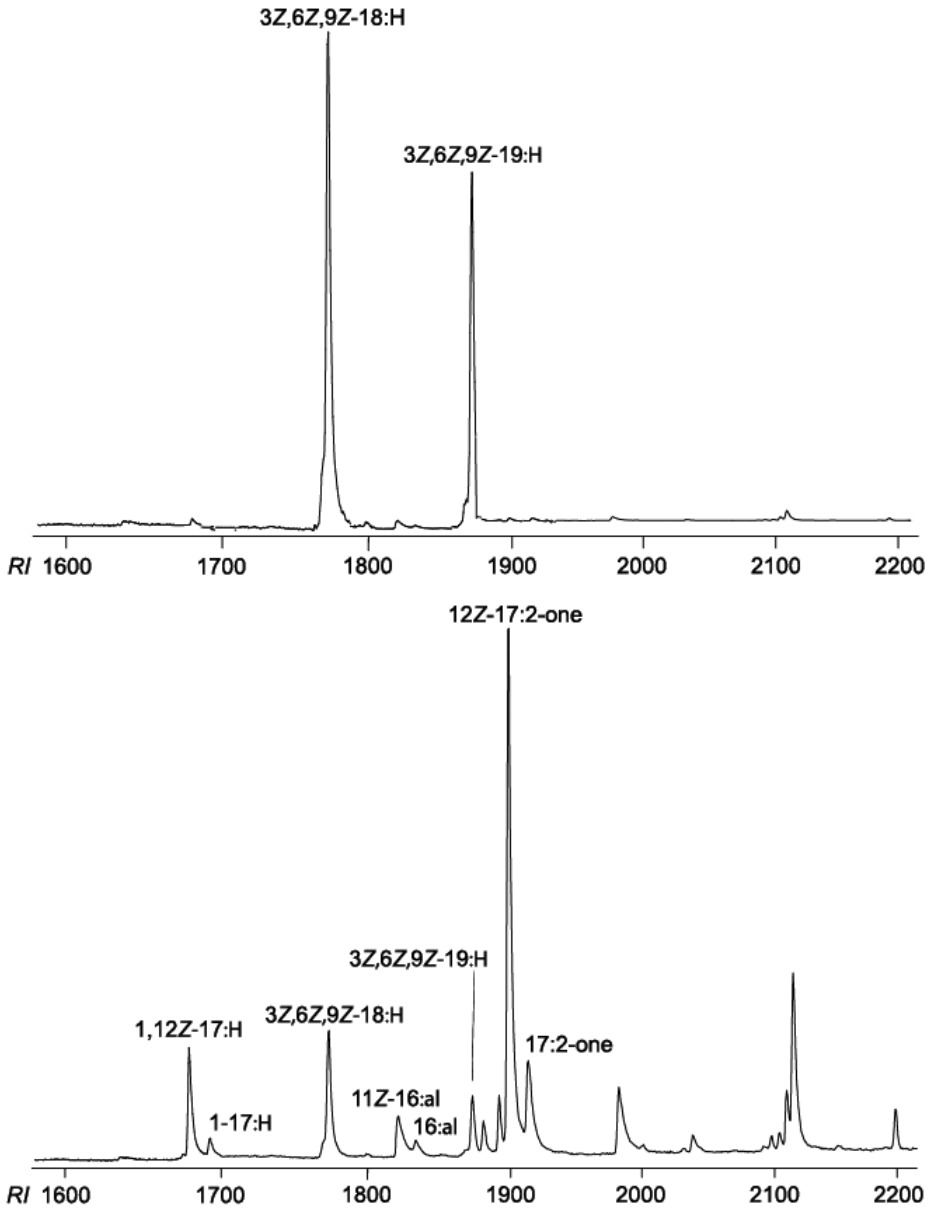

Figure 99: Comparison of contents of the pheromone gland of *Erannis bajaria* females in the year 1996 (above) and year 2006 (below). Parts of total ion chromatograms between *RI* 1600 and 2200 are shown.

While the concentration of 3*Z*,6*Z*,9*Z*-18:H and 3*Z*,6*Z*,9*Z*-19:H was reduced, the blend contained

compounds resembling more type I pheromones. The blend was mainly characterized by (*Z*)-1,12-heptadecadiene (1,12*Z*-17:H), 1-heptadecene (1ene-17:H), (*Z*)-11-hexadecenal (11*Z*-16:al), hexadecanal (16:al), (*Z*)-12-heptadecen-2-one (12*Z*-17:2-one) and 2-heptadecanone (17:2-one). 12*Z*-17:2-one represented the main compound in this blend with 39.0%. The presence of 1ene-17:H, 16:al, and 17:2-one in this blend showed that the compounds are putatively precursors in the biosynthesis of 1,12*Z*-17:H, 11*Z*-16:Al, and 12*Z*-17:2-one. The trienes 3*Z*,6*Z*,9*Z*-18:H and 3*Z*,6*Z*,9*Z*-19:H contributed 10.2% and 4.2% to the TIC. Table 15 shows the comparison between the contents of the PG blend in the year 1996 and 2006.

Table 15: Comparison between the composition of a pheromone gland extract of calling *Erannis bajaria* in the year 1996 and 2006. Intensities of compounds are given in %. This compilation summarizes the compounds which were identified in all investigated samples (10 extracts).

RI	Compound	PG 1996	PG 2006
1683	1,12Z-17:H	-	8.0
1695	1ene-17:H	-	1.6
1775	3Z,6Z,9Z-18:H	50.0	10.2
1815	11Z-16:al	-	4.4
1826	16:al	-	1.7
1875	3Z,6Z,9Z-19:H	50.0	4.2
1893	1ene-19:H	-	3.5
1898	12Z-17:2-one	-	39.0
1910	17:2-one	-	11.1
2032	18:al	-	1.8
2100	21:H	-	0.7
2113	19:2-one	-	11.8
2200	22:H	-	2.0

These structures were elucidated by mass-spectral analysis, microderivatizations, and synthesis. Those species of lepidoptra exhibiting a mixed composition of type I and type II structures in the PG are rare. Recently, 11*Z*-16:Al and 3*Z*,6*Z*,9*Z*-23:H were reported as sex pheromone components of the red banded mango caterpillar *Deanolis sublimbalis* [Gibb *et al*., 2007].

4.3.2 Structure elucidation of the novel compounds

The mass spectrum of 1,12*Z*-17:H is shown in Figure 100 and this compound was characterized by a *RI* of 1683.

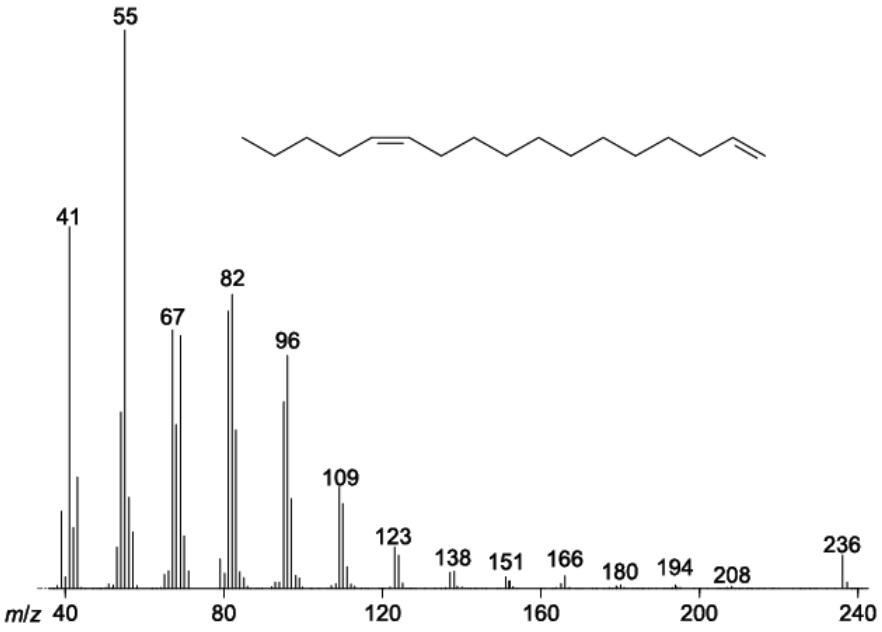

Figure 100: Mass spectrum of (*Z*)-1,12-heptadecadiene.

The ion *m*/*z* = 55 represented the base ion in this mass spectrum and furthermore, the ions *m*/*z* = 41, 67, 82, 96, and 123 were of importance. Based on its molecular weight and a slight lower *RI* than 1ene-17:H, a heptadecadiene with unknown location of the double bonds was proposed.

The position of the double bonds were tried to elucidate by DMDS-derivatization; the corresponding mass spectrum is shown in Figure 101.

The mass spectrum was characterized by the molecular ion 424, the base ion *m*/*z* = 259, and the ion *m*/*z* = 117. The ion *m*/*z* = 117 indicated a fragment with the formula $C_6H_{13}S^+$ and the base ion was formed as counter ion after elimination of thiomethanol as expected for a diene with a double bond in position 12. Besides, elimination of thiomethanol demonstrated the presence of a second double bond in this fragment. But its location remained unclear because characteristic fragment ions were lacking. Therefore, the second double was assumed at position 1. This proposal was confirmed by the synthesis of 1,12*Z*-17:H and its mass spectrum and that of the corresponding DMDS-derivative as well as their *RI*s (3260 for the DMDS-derivative) were identical with the data of the natural compound. This compound, **108,** was synthesized by a Wittig-reaction with 11*Z*-16:al in 63% yield. The (Z)- and the (*E*)-isomer were base-line separated under the applied conditions in GC and the (*E*)-isomer eluted shortly after the (*Z*)-isomer.

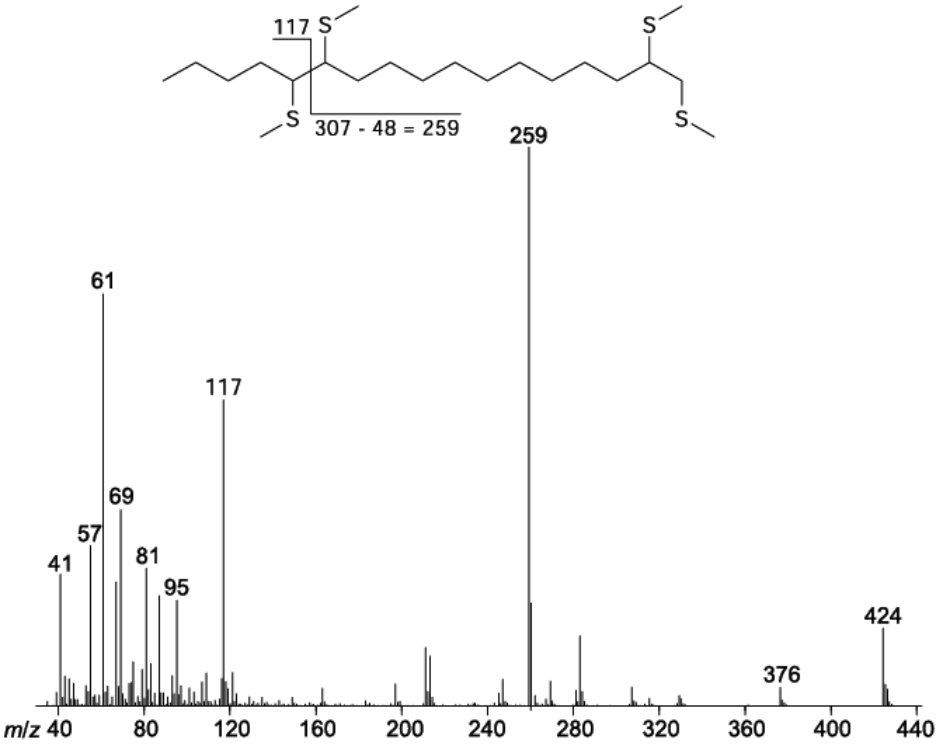

Figure 101: Mass spectrum of the corresponding DMDS-derivative of (*Z*)-1,12-heptadecadiene.

The mass spectrum of 11*Z*-16:al is illustrated in Figure 102 and a *RI* of 1815 was determined for this compound. The mass spectrum of the corresponding DMDS-derivative is shown in Figure 103.

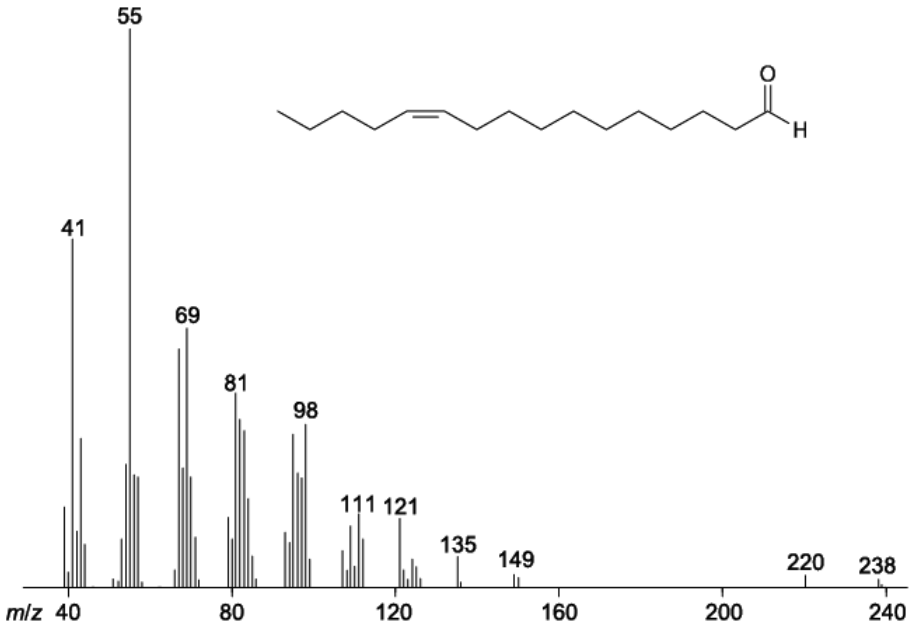

Figure 102: Mass spectrum of (*Z*)-11-hexadecenal.

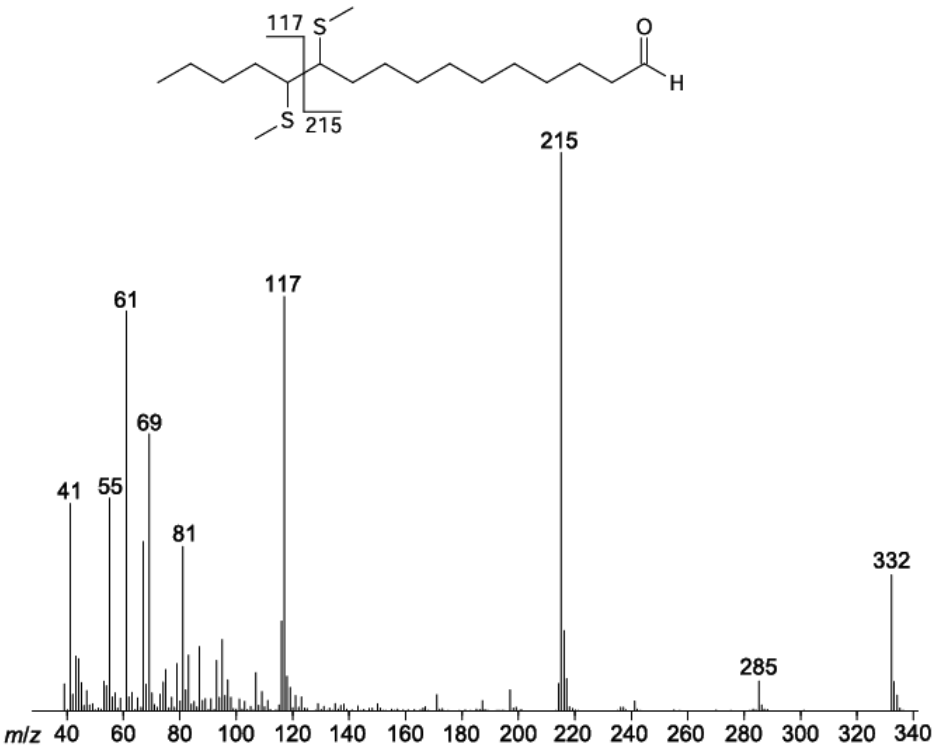

Figure 103: Mass spectrum of the corresponding DMDS-derivative of (*Z*)-11-hexadecenal.

A double bond in position 11 was elucidated by the ions *m*/*z* = 117 and 215. A hypothetical double bond in position 4, also leading to these ions, was ruled out because the *RI* of the synthetic compound and the correpsonding DMDS-derivative (*RI* = 2516) and also their mass spectra were identical to those of the natural compound. Furthermore, the (*E*)-configuration was excluded because both compounds were separated in GC under the applied conditions.

Analogously, the structure of 12*Z*-17:2-one was elucidated. The mass spectrum of this compound and that of the corresponding DMDS-derivative are shown in Figure 104 and 105. This compound was characterized by a *RI* of 1898 and eluted shortly before 17-2:one. The double bond in position 12 was indicated by the ions *m*/*z* = 117 and 229 and this derivative was characterized by a *RI* of 2601. A double bond in position 4 was excluded because all data of the natural compound were identical to those of a synthetic reference of 12*Z*-17:2-one **111**. This compound was synthesized by treating 11*Z*-16:Al **109** with methylmagnesium chloride to furnish the 2-alcohol **110** in 92% yield followed by oxidation with PCC to provide **111** in 94% yield (Figure 106). The (*Z*)-configuration was confirmed because both diastereoisomers were base-line separated under used GC-conditions.

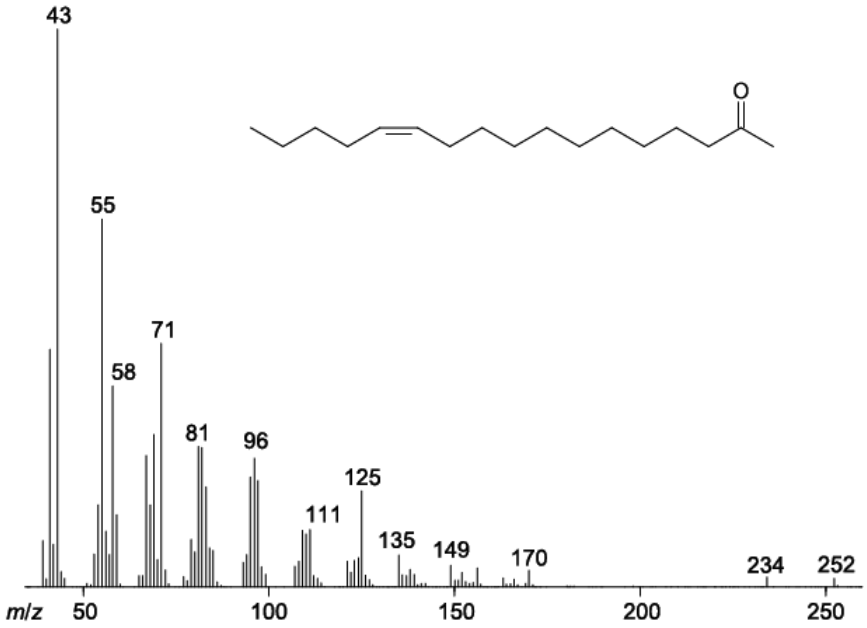

Figure 104: Mass spectrum of (*Z*)-12-heptadecen-2-one.

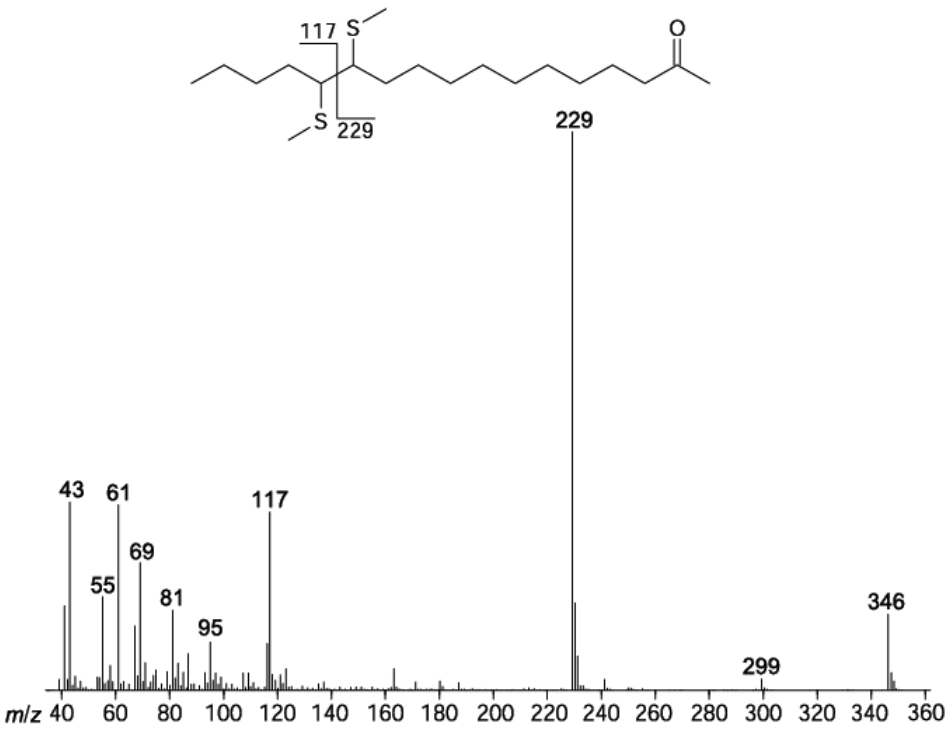

Figure 105: Mass spectrum of the corresponding DMDS-derivative of (*Z*)-12-heptadecen-2-one.

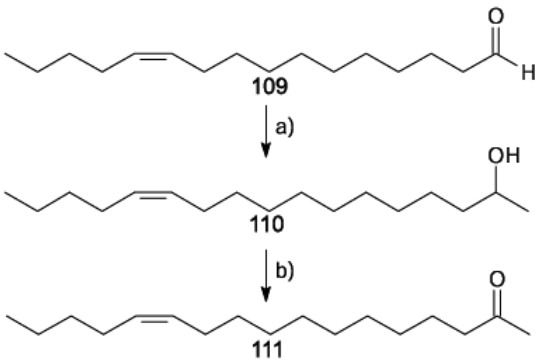

Figure 106: Synthesis of (*Z*)-12-heptadecen-2-one. a) MeMgCl, THF, rt overnight, 94%; b) PCC, CH_2Cl_2, rt overnight, 94%.

Field experiments with the new compounds are planned in the fall flying period of *E. bajaria* to investigate if these novel compounds are involved in the semiochemistry of this species and if the previous elucidated pheromones are still functional.

5. The catalepsis pheromone of the male desert spider *Agelenopsis aperta* and a female pheromone of the wasp spider *Argiope bruennichi*

5.1 Spider pheromones

The first spider pheromone was identified in *Linyphia triangularis* inducing web reduction [Schulz and Toft, 1993]. After perception of the chemical cue, the male enters the web of the unmated female and starts to roll up her web. Obviously, the web reduction behaviour prevents the attraction of other conspecific males therefore ensuring male mating success of the first arriving male. This pheromone was identified by GC-MS investigations of silk extracts. The condensation product of two molecules of (*R*)-3-hydroxybutyric acid was shown to be the active compound. Its AC was determined by chiral GC with reference compounds. Web reduction is also induced by the monomer. The dimer is unstable and decomposes slowly into (*R*)-3-hydroxybutyric acid and crotonic acid and both compounds attracted males in laboratory assays [Toft and Schulz, unpublished data]. Based on these results, a dual function for the dimer was assumed. The dimer induces the web reduction behaviour and reflects a propheromone because the degradation products (*R*)-3-hydroxybutyric acid and crotonic acid trigger male attraction. This pheromone system is also applied by the related species *L. tenuipalpis*, living in the same habitat, and other linyphiid species.

Females of the desert spider *Agelenopsis aperta* release 8-methyl-2-nonanone for male attraction [Papke *et al*., 2001]. This compound was present in headspace extracts of virgin females two weeks after final moult. This compound attracted males and induced courtship behaviour in bioassays independent of the presence of a receptive female, in contrast to other behavioural traits. Doses of the ketone in the range of 500 ng were sufficient for the attraction of males in these experiments.

Cupilure, (*S*)-dimethyl citrate, was identified as the female sex pheromone in the ctenid spider *Cupiennius salei* [Tichy *et al.*, 2001]. The pheromone is deposited on the female silk and males respond with vibrational cues after perception of the chemical cue followed by display of other courtship traits. The active compound was isolated from the silk of virgin females by addition of deuterated methanol and subsequent ^{1}H-NMR-analysis of the resulting extract. The AC of the natural product was determined by chiral GC after synthesis of racemic and enantiopure dimethyl citrate. This compound occurred also on the silk of closely related species as in *C. getazi* and *C. coccineus* [Papke, Schulz, and Tichy, unpublished data].

5.2 The catalepsis pheromone of *Agelenopsis aperta* males

5.2.1 Previous investigations

In general, male spiders are smaller than their conspecifics females. Based on the smaller body size, males befall agression during or after copulation often followed by the death of the male spider.

But males developed strategies to increase their viability. Vibratory signals are putatively applied to reduce the mortality rate of males in the sheet-line weaving spider *Frontinella pyramitea* [Suter and Hirschheimer, 1986]. Males in the genus *Tetragnatha* have longer chelicerae than the females and these are used to dominate the shorter chelicerae of conspecific females during copulation resulting in a decreased mortality rate [Bristowe, 1976]. Nuptial gifts represent another male strategy. Prey items are offered to females in the fishing spider *Pisaura mirabilis* followed by accomplishing the insemination during the female occupies on the prey [Lang, 1996].

Males of the funnel-web spider *A. aperta* induce a quiescent state in females [Gering, 1949]. First, communication proceeds via vibratory signals and then the male moves to the receptive female, mounts her, followed by induction of catalepsis. Further investigations demonstrated that the catalepsis do not rely inevitably on physical contact between the sexes and a distance of 5 cm is sufficient. Based on these experiments, a volatile compound emitted by males was proposed to be responsible for the quiescent state in females. Experiments with male tissue homogenates supported this proposal. Air passed over male tissue homogenates and was directed to the female and catalepsis was induced in 58.3% of the tested females in these trials. Similarly, males of the cockroach *Nauphoeta cinera* release two pheromones [Moore *et al*., 1995]. The first one serves as attractant for females and the second one is an aphrodisiac. Furthermore, a male aphrodisiac, picked up by females during copulation, was described in theridiid spiders of the genus *Argyrodes* [Legendre and Lopez, 1974].

The elucidation of compounds responsible for the induction of catalepsis in females of *A. aperta* was the aim of this study. Analyses were performed by collection of headspace extracts of *A. aperta* individuals. The chemical composition of the headspace of a virgin female, virgin female/mature male, mature male/web of a virgin female, and male alone were investigated. Individuals of *A. aperta* were encaged in a glass chamber for this purpose. A mesh barrier was used in the virgin female/mature male to prevent physical contact. Each experiment was repeated three times. This comparative study was performed to elucidate compounds only emitted by males or formed preferentially by males in the presence of a virgin female or her web. Furthermore body extracts of the abdomen, the cephalothorax, the pedipalps, and the legs of male *A. aperta* were

analysed.

5.2.2 Investigation of male body extracts of *Agelenopsis aperta*

The extracts of the cephalothorax, abdomen, pedipalps, and legs consisted predominantly of *n*-alkanes as well as their momomethyl and dimethyl branched isomers. The profile of the extracts was very similar and thus merely the GC-profile of the cephalothorax extract is shown in Figure 107. Table 16 summarizes the composition of the investigated extract.

Table 16: Chemical composition of body extracts of male *Agelenopsis aperta*. ct = cephalothorax, a = abdomen, p = pedipalps, l = legs. Intensities of compounds are given in %.

RI	Compound	ct	a	p	l
981	Benzaldehyde	2.4	0.5	-	-
1827	16:al	Tr	-	-	-
2032	18:al	0.2	-	-	1.6
1792	Xene-18:H	0.3	-	-	-
-	9*Z*,12*Z*-18:COOH	21.5	-	-	-
1970	Xene-20:H	1.6	-	-	-
2061	1,3-20:diol	1.0	-	-	-
2146	1,3-21:diol	0.6	-	-	-
2239	1,3*S*-22-diol	1.6	-	-	-
2614	22:OAc	0.2	-	-	-
2663	2Me-26:H	0.2		0.3	0.1
2700	27:H	0.7	0.7	0.6	1.3
2729	11/13Me-27:H	-	0.5	Tr	0.2
2763	2Me-27:H	0.2	-	0.3	0.1
2772	3Me-27:H	-	0.5	-	-
2800	28:H	0.7	1.7	1.3	2.6
2829	11/12/13/14Me-28:H	0.2	-	0.3	0.2
2864	2Me-28:H	5.8	8.9	16.1	20.3
2885	Xene-29:H	0.1	-	-	-
2900	29:H	1.3	3.4	3.2	7.4
2929	11/13/15Me-29:H	1.3	2.2	2.8	2.5
2938	7Me-29:H	0.2	0.2	0.3	-
2947	5Me-29:H	0.1	0.2	0.3	0.3
2961	2Me-29:H	0.7	0.7	0.6	0.8
2972	3Me-29:H	0.5	1.0	0.6	0.9
3000	30:H	0.5	0.5	0.3	0.5
3029	12/13/14/15Me-30:H	0.9	-	0.3	0.8
3063	2Me-30:H	4.9	8.4	10.1	16.5
3100	31:H	-	-	1.9	4.0
3114	Cholesterol	27.9	29.7	-	-

RI	Compound	ct	a	p	l
3129	9/11/13/15Me-31:H	4.2	-	6.6	4.0
3149	11,15/11,17/11,19DiMe-31:H	2.3	-	3.8	0.5
3158	9,15DiMe-31:H	-	-	„	0.8
3165	7,17DiMe-31:H	-	-	„	-
3172	5,15DiMe-31:H	0.2	-	0.3	-
3183	Ac13:18OH	0.5	1.0	1.6	1.9
3200	32:H	0.3	-	0.9	-
3226	12/13/14/15/16Me-32:H	1.5	2.7	2.5	1.0
3248	13,17DiMe-32:H	0.7	1.5	1.5	0.4
3261	2Me-32:H	0.3	-	0.9	0.4
3281	Ac14:18OH	0.2	-	0.9	0.5
3327	11/13/15/17Me-33:H	3.6	7.6	7.1	7.2
3348	13,17/13,19/15,17/15,19DiMe-33:H	2.4	4.7	5.6	4.7
3379	Ac13:20OH	0.6	2.5	3.4	3.0
3400	34:H	0.1	-	-	-
3426	12/13/14/15/16/17Me-34:H	0.8	2.4	2.4	4.7
3447	13,19/15,19/13,21DiMe-34:H	1.1	2.7	3.4	3.0
3481	Ac12:22OH	0.1	-	1.5	0.5
3529	11/13/15/17Me-35:H	1.6	5.7	4.9	1.9
3548	13,17/13,19/15,19DiMe-35:H	2.7	6.6	8.4	4.8
3581	Ac13:22OH; Ac15:20OH	0.5	3.0	4.9	0.7
3600	36:H	0.1	-	-	-
3645	13,19/15,19DiMe-36:H	0.2	-	-	-
3724	13/15/17/19Me-37:H	0.1	-	-	-
3741	15,19/15,21DiMe-37:H	0.2	-	-	-

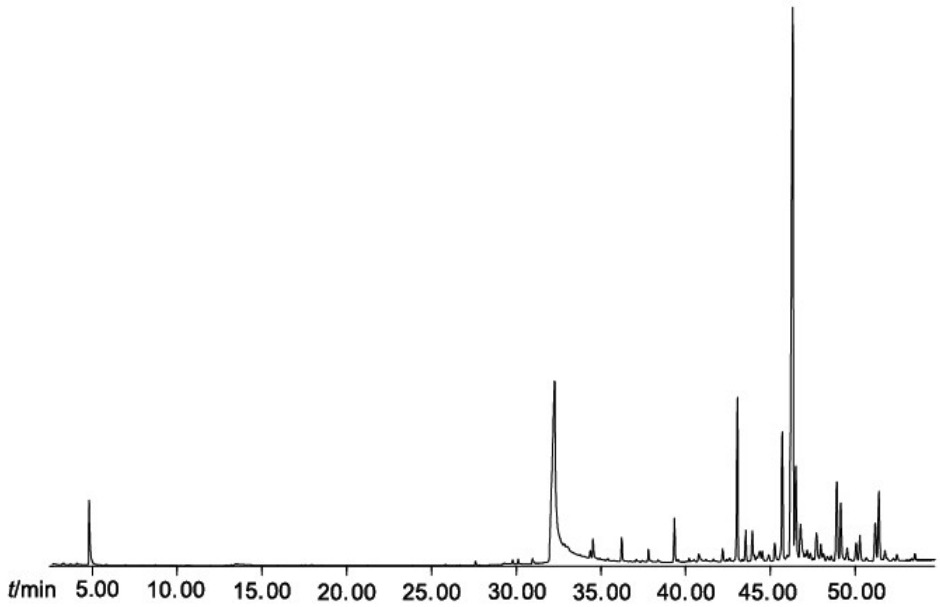

Figure 107: Total ion chromatogram of a cephalothorax extract of *Agelenopsis aperta* males.

Alkanes accounted 40.6% to the TIC. The alkane blend was subdivided into 3.7% *n*-alkanes, 27.1% monomethyl branched alkanes, and 9.8% dimethyl branched alkanes. The methyl group in the monomethyl branched alkanes were internally arranged on odd-numbered carbon atoms in odd-numbered alkanes and on even-numbered carbon atoms in even-numbered representatives. Besides, this blend contained terminal branched derivatives with the methyl branch in 2- or 3-position. Methyl groups in dimethyl branched alkanes occurred internally in odd-numbered positions.

Wax esters were also present in the lipid extracts, but their structures were not elucidated because of their minute amounts. After conversion into other derivatives (pyridylmethyl esters, nicotinic acid esters), their recovery was impaired. Based on the mass spectra of the wax esters, the presence of 2-methylalkanoic acids was indicated by the disinct ion $m/z = 74$. 2-Methylalkanoic acids with 12 to 14 cabon atoms conjugated to alcohols with 18, 20, or 22 carbon atoms were present in these extracts.

Furthermore, 1,3-alkanediols with 20, 21, and 22 carbon atoms, also known as components of other spider lipid blends and those of *Heliconius* butterflies, were identified in the cephalothorax extract based on a striking ion $m/z = 131$ in the corresponding mass spectra. These diols form cyclic dimethylsiloxane derivatives on a BPX-5-phase and the ion $m/z = 131$ results from a cleavage in α-position to the dimethylsiloxane moiety. The AC of 1,3-docosanediol (1,3-22:diol) was elucidated after derivatization of the natural extract with (*R*)-dichloro-ethyl-1-(phenylethyl)silane followed by GC-analysis on an achiral BPX-5-phase. Coelution of the corresponding derivative of the natural sample and that of the (*S*)-enantiomer proved the (*S*)-configuration of the natural product.

Benzaldehyde occurred as only volatile compound in the extracts of the cephalothorax (2.4%) and the abdomen (0.5%). But this compound was absent in extracts of legs and pedipalps. It was of interest because its volatily matches with the criterion expected for the catalepsis pheromone. This compound was also present in every trial during the headspace experiments. Benzaldehyde is a widespread natural compound in arthropods and there are known examples in which benzaldehyde was described as allomone, pheromone, or kairomone [pherobase].

The abdominal extract composed of 62.3% alkanes and those were subdivided in 6.3% *n*-alkanes, 40.5% monomethyl branched alkanes, and 15.5% dimethyl branched alkanes. Alkanes contributed 85.7% and 91.9% to the TIC of extracts of the pedipalps and legs. Mainly, monomethyl branched alkanes occurred and the most abundant representative was 2-methyloctacosane with 16.1% and 20.3%. Monomethyl branched alkanes were found in 56.4% and 61.9%, respectively. This compound was also the most important alkane in the cephalothorax extract. Dimethyl branched

alkanes accounted for 23.0% in the pedipalp extracts and 14.2% in the leg extracts and their chain length ranged from 31 to 35 carbon atoms. *n*-Alkanes had an amount of 6.3% in the extracts of the pedipalps and 15.8% in the leg extracts.

5.2.3 Head space experiments with *Agelenopsis aperta*

Four experiments were performed. First, a mature male was arrested in the glass chamber and the headspace was collected over a period of 16 to 20 hours on a carbon filter. Second, the headspace of a virgin *A. aperta* female was collected. Third, a mature male was confronted with the web of a virgin female and the headspace was also trapped over a period of 16 to 20 hours. This web was constructed by a virgin female one day before the experiment started, followed by removal of the female. Fourth, a mature male and a virgin female, separated by a mesh, were encaged in the glass chamber and the headspace was collected over the same period. Each individual was used only once in these experiments. The composition of the headspace extracts is summarized in Table 17.

Table 17: Chemical composition of *Agelenopsis aperta* headspace extracts. m = male alone, mf = male and female, mfw = male and female web, f = female alone. Tr = < 0.1%, + = < 1%, ++ = 1 – 3%, +++ = > 3%. uk indicates unknown compounds.

RI	Compound	m	mf	mfw	f
821	6:al	-	+	Tr	Tr
905	7:2one	-	+	-	-
915	7:al	+	+	+	-
938	γ- Butyrolactone	+	+	Tr	-
975	4-Methylbutyrolactone	-	-	Tr	-
981	Benzaldehyde	+	+	+	+
996	Sulcatone	++	+++	++	+
1001	8:2one	-	Tr	Tr	-
1015	8:al	++	++	++	+
1056	4-Methyl-4-ethinyl-butyrolactone	+	+	-	-
1065	*N*-3-methylbutylpyrrole	+	++	+	+
1073	4-Ethylbutyrolactone	Tr	+	-	-
1076	δ-Valerolactone	Tr	+	Tr	-
1080	7Me-8:al	+	+	+	-
1094	6Me-8:al	-	Tr	-	-
1101	9:2one	-	+	-	Tr
1115	9:al	+++	+++	+++	++
1151	*N*-(3-methylbutyl)acetamide	+	Tr	Tr	-

RI	Compound	m	mf	mfw	f
1160	5-Methyl-2-pentyl-furan	Tr	Tr	Tr	-
1171	3-Methyl-*N*-3-methylbutylpyrrole	+	++	+	+
1173	4-Propylbutyrolactone	-	Tr	-	-
1173	2*E*-9:al	+	+	Tr	-
1179	8Me-9:al	+	+	+	Tr
1200	12:H	-	+	-	Tr
1201	10:2one	-	+	-	Tr
1205	5-Ethylvalerolactone	-	Tr	-	-
1216	10:al	+++	+++	+++	++
1266	uk; 55 (100), 70, 83, 98, 125	+	+	Tr	-
1275	4-Butylbutyrolactone	Tr	+	Tr	-
1287	uk, 41 (100), 55, 70, 81, 97	-	-	-	+
1288	8Me-10:al	+	+	+	-
1291	uk; 43 (100), 69, 83, 98	Tr	+	+	-
1300	13:H	+	Tr	+	Tr
1300	uk; 81, 94,109 (100), 122, 136, 165	Tr	Tr	-	-
1301	11:2one	-	Tr	-	-
1303	5-Propylvalerolactone	-	Tr	-	-
1313	uk; 41 (100), 55, 68, 81, 98	+	++	++	-
1317	11:al	++	++	+	+
1330	uk; 53, 81, 96,109 (100), 165	Tr	+	+	-
1364	3,6-decanedione	Tr	+	Tr	-
1372	uk; 43 (100), 57, 72	-	+	+	Tr
1374	2*E*-11:al	Tr	+	-	-
1380	4-Pentylbutyrolactone	-	+	Tr	Tr
1383	uk; 43 (100), 61, 69, 84, 150, 210	-	+	+	Tr
1386	uk; 43 (100), 67, 81, 107, 150	Tr	++	++	Tr
1391	1-Dodecen-3-one	Tr	Tr	Tr	-
1395	uk; 43 (100), 69, 84	Tr	+	+	-
1408	5-Butylvalerolactone	-	+	Tr	-
1419	12:al	++	++	+	+
1457	Geranylacetone	+++	+++	+++	++
1479	2*E*-12:al	+	+	+	-
1486	4-Hexylbutyrolactone	Tr	+	+	-
1500	15:H	+	+	+	+
1504	13:2one	Tr	Tr	Tr	+
1522	13:al	+	+	+	+
1533	uk; 41 (100), 69, 93	Tr	+	+	-
1539	12:formate	Tr	+	Tr	-
1582	2*E*-13:al	-	+	Tr	-
1600	16:H	-	Tr	Tr	-
1622	14:al	+	+	+	++

RI	Compound	m	mf	mfw	f
1694	Hexylsalicylate	+	+	+	Tr
1700	17:H	+	+	+	++
1706	15:2one	Tr	+	Tr	Tr
1725	15:al	Tr	+	+	Tr
1742	14:formiate	Tr	Tr	Tr	-
1751	Farnesal	-	Tr	Tr	-
1800	18:H	+	+	Tr	++
1808	16:2one	-	Tr	-	-
1843	Farnesylacetaldehyde	++	+++	+++	++
1900	19:H	-	Tr	-	-

Several compound classes were identified in the headspace extracts based on interpretation of mass spectra, evaluation of *RI*s, and comparison with reference mass spectra and those of synthetic compounds. Parts of TICs of these experiments are illustrated in Figure 108.

Virgin females emitted volatiles in a smaller amount than mature males. Female- and male-derived compounds were observed during these experiments but compounds of unknown origin occurred also. Anyway, all male compounds in greater intensity were considered as interesting compounds concerning the catalepsis.

Unbranched aldehydes were strongly present in the headspace extracts and they were male- and also female-derived. Nonanal (9:al) and decanal (10:al) were especially prominent in these experiments and the latter one reflected the most abundant compound in all of the experiments. These derivatives were elucidated by comparison with reference spectra and *RI*s. Furthermore, (ω-1)- and (ω-2)-aldehydes were identified as components of the headspace. Except of 8-methylnonanal (8Me-9:al), 7-methyloctanal (7Me-8:al), 6-methyloctanal (6Me-8:al), and 8-methyldecanal (8Me-10:al) were absent in the female experiment. These structures were also elucidated by comparison with reference mass spectra and their corresponding *RI*s [Tajima *et al*., 1990].

In addition, pyrroles and an irregular terpene aldehyde were identified in these headspace extracts as side components. These compounds occurred in all trials but they were produced in greater amounts in males and in the other male-involved trials than by females alone. The irregular terpene was an aldehyde with a bis-homo sesquiterpene skeleton and was characterized by the mass spectra in Figure 109. A small ion m/z = 248 exhibited the molecular ion of this compound and comparison with reference mass spectra indicated farnesylacetaldehyde as structure for this compound. A *RI* of 1843 was determined for this compound. A synthetic reference was prepared according to Figure 110. All-*trans*-farnesylchloride **112** was alkylated with deprotonated acetonitrile to

farnesylacetonitrile **113** in 32% yield followed by reduction with DIBAL in 91% yield to furnish farnesylacetaldehyde **114**. The *RI* of this compound as well as its mass spectrum were identical with those of the natural compound.

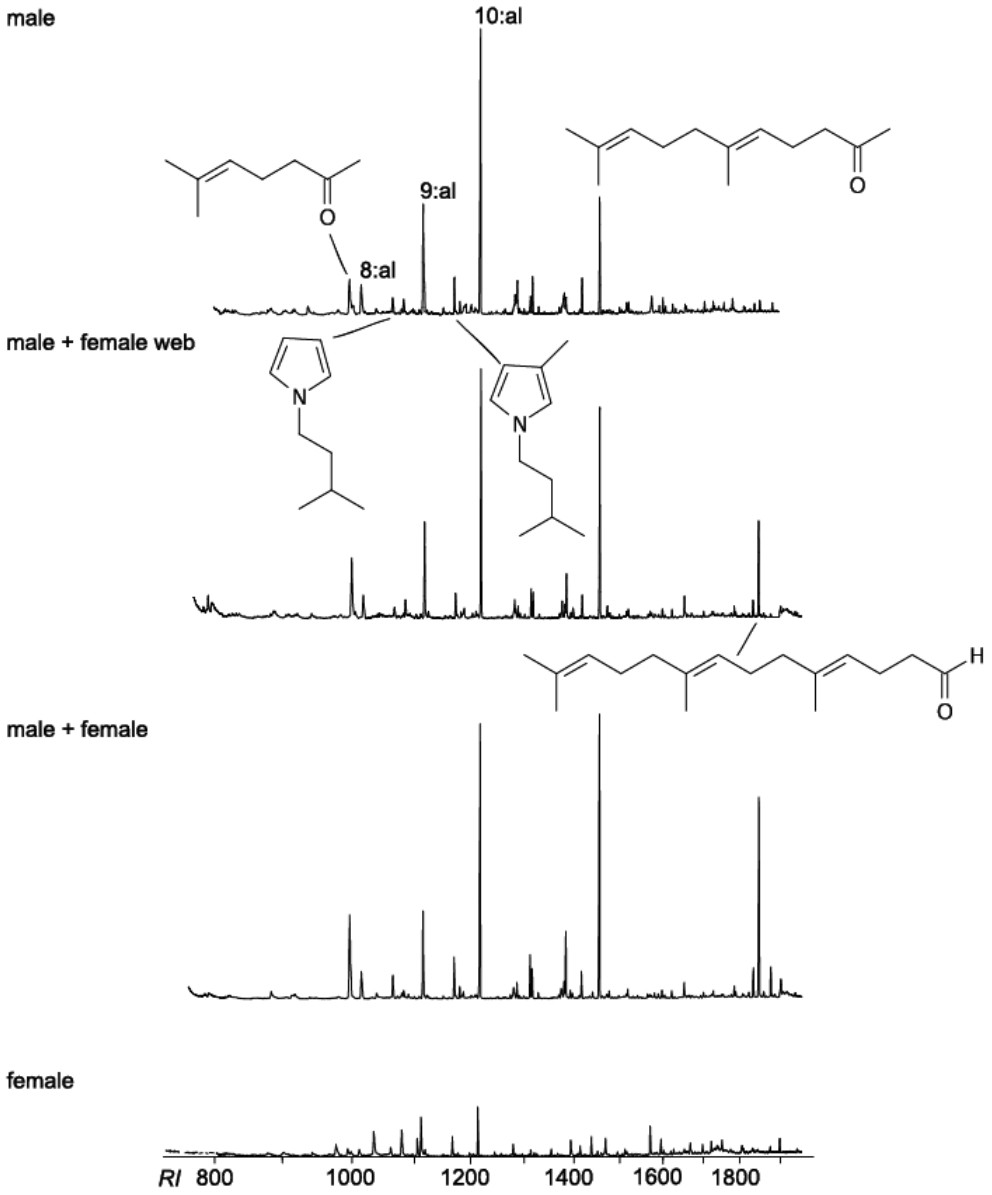

Figure 108: Total ion chromatograms of headspace experiments with *Agelenopsis aperta*. First: male alone; second: male and female web; third: male and female; fourth: female alone.

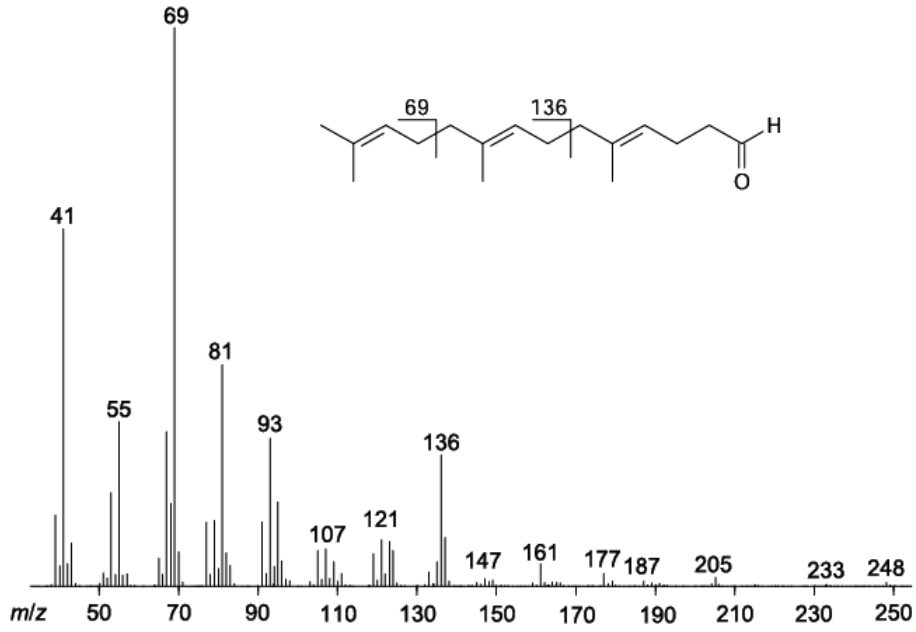

Figure 109: Mass spectrum of farnesylacetaldehyde.

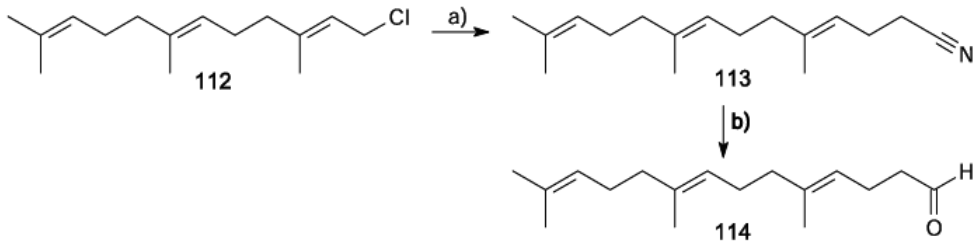

Figure 110: Synthesis of all-*trans*-farnesylacetaldehyde. a) *n*-BuLi, CH_3CN, 2h -78°C, 32%; b)DIBAL, -78°C → rt, 91%.

The biosynthesis of this irregular terpene is thought to proceed about the elongation of the ubiquitous sesquiterpene precursor farnesylpyrophosphate **120** according to Figure 111.

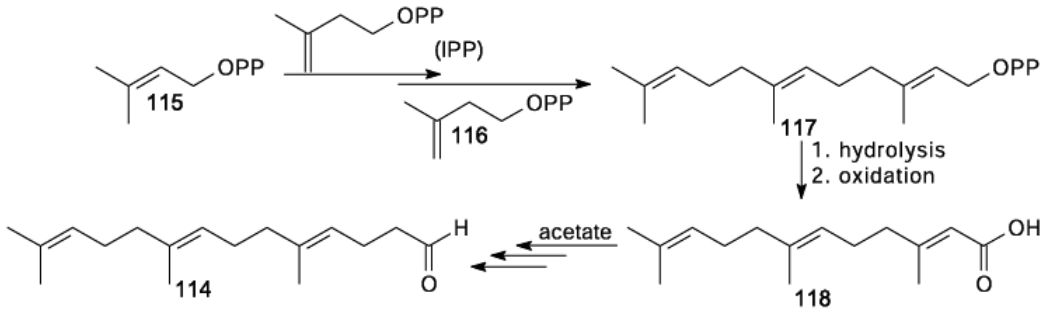

Figure 111: Proposed biosynthetic pathway for the formation of all-*trans*-farnesylacetaldehyde.

First, **117** is formed in a standard pathway by the addition of two *iso*-pentenylpyrophosphate units

(IPP) **116** to dimethylallylpyrophosphate (DMAPP) **115**. Hydrolysis of **117**, followed by oxidation furnishes the corresponding acid **118**. Then, chain elongation takes place by the addition of acetate via malonate to provide the corresponding acid which is finally reduced to farnesylacetaldehyde **114**.

One of the two pyrroles was *N*-alkylated with the molecular weight of 137 amu and based on its molecular weight an alkyl residue with 5 carbon atoms was concluded (Figure 112).

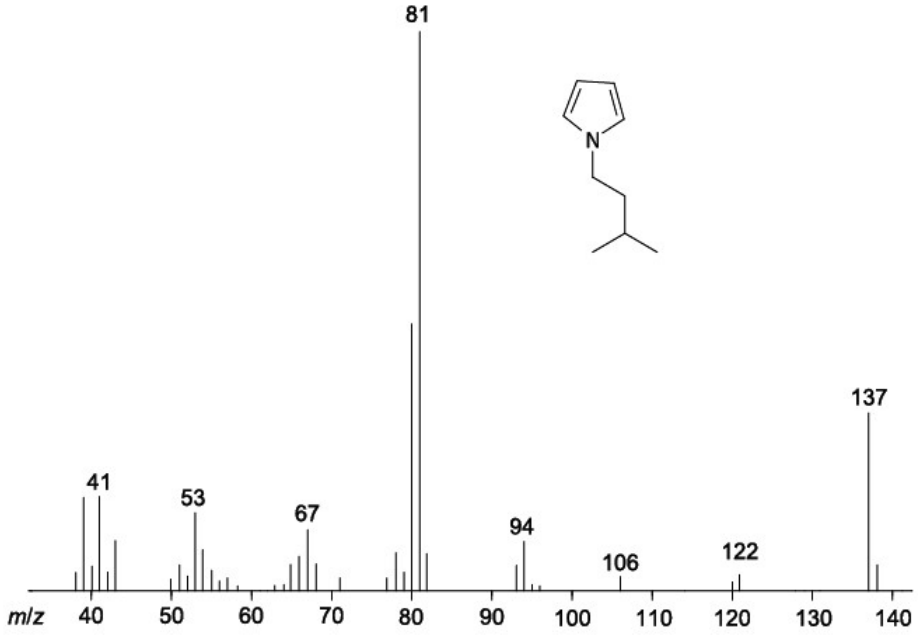

Figure 112: Mass spectrum of *N*-3-methylbutylpyrrole.

The ion *m*/*z* = 81 represented the base ion and was formed by an α-cleavage of the alkyl chain. The absence of the ion *m*/*z* = 108 suggested the pyrrole to be alkylated with a 3-methylbutyl residue. In contrast, this ion was present in the mass spectrum of *N*-pentylpyrrole. Furthermore, the *RI* of 1105 for *N*-pentylpyrrole deflected from that of the natural one with 1066. Synthetic references of *N*-3-methylbutylpyrrole **119** and *N*-pentylpyrrole **120** were prepared by alkylation of pyrrole in 60% and 80% yield, respectively [Brockmann and Tour, 1995]. The mass spectral and chromatographic properties of the 3-methylbutyl derivative were identical to those of the natural compound. This compound was described as constituent of the okra plant *Hibiscus esculentus* besides of other pyrrole derivatives [Ames and MacLeod, 1990]. The immature green pods of okra are used as a vegetable source and okra is a cosmopolitan plant with origin in Africa.

The second pyrrole derivative with a molecular weight of 151 amu was characterized by the mass spectrum in Figure 113.

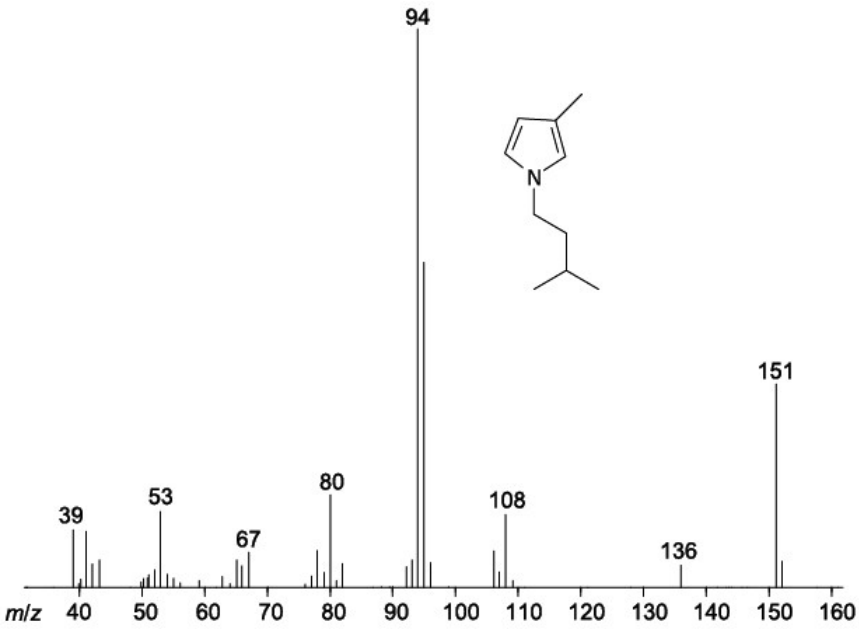

Figure 113: Mass spectrum of 3-methyl-*N*-3-methylbutylpyrrole.

The base ion in the mass spectrum was represented by the ion *m*/*z* = 94 and the cleavage of a alkyl residue with 5 carbon atoms from a methylated pyrrole unit was proposed to evoke this fragment. Like before, the presence of a 3-methylbutyl moiety was assumed because the ion *m*/*z* = 122 was not present in the mass spectrum. The location of the methyl group could not be deduced by mass spectral analysis but was clarified by synthesis of 2-methyl-*N*-3-methylbutylpyrrole **123** according to Figure 114.

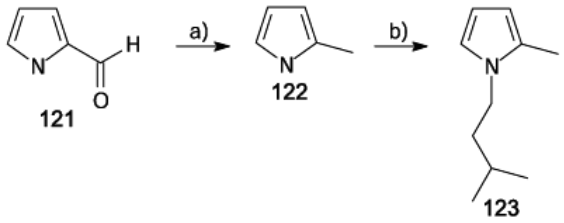

Figure 114: Synthesis of 2-methyl-*N*-3-methylbutylpyrrole. a) LAH, 48h reflux, 40%; b) NaOH, tetrabutylammonium iodide, 3-methylbutyl bromide, NaOH, reflux 24h, 17%.

pyrrole-2-carbaldehyde **121** was reduced to 2-methylpyrrole **122** in 40% yield [Liddel *et al.*, 1993]. Finally, **122** was alkylated with 3-methylbutyl bromide in 17% yield to furnish **123**. The mass spectrum was identical to that of the natural compound but the *RI*s of natural compound and synthetic reference were different. The synthetic reference was characterized by a *RI* of 1148 in contrast to the *RI* of 1171 for the natural compound. Based on these results, methylation in

3-position was concluded for the natural compound and this compound is unknown so far from a natural source.

The biosynthesis of the pyrrole derivatives seems to be closely related because they differ only in one methyl group. In general, modified amino acids (AAs) are proposed as precursors for pyrrole biosynthesis. Taking into account well-established key steps in amino acid and alkaloid biosynthesis, the formation of the pyrroles might proceed as shown in Figure 115 [Dewick, 2006].

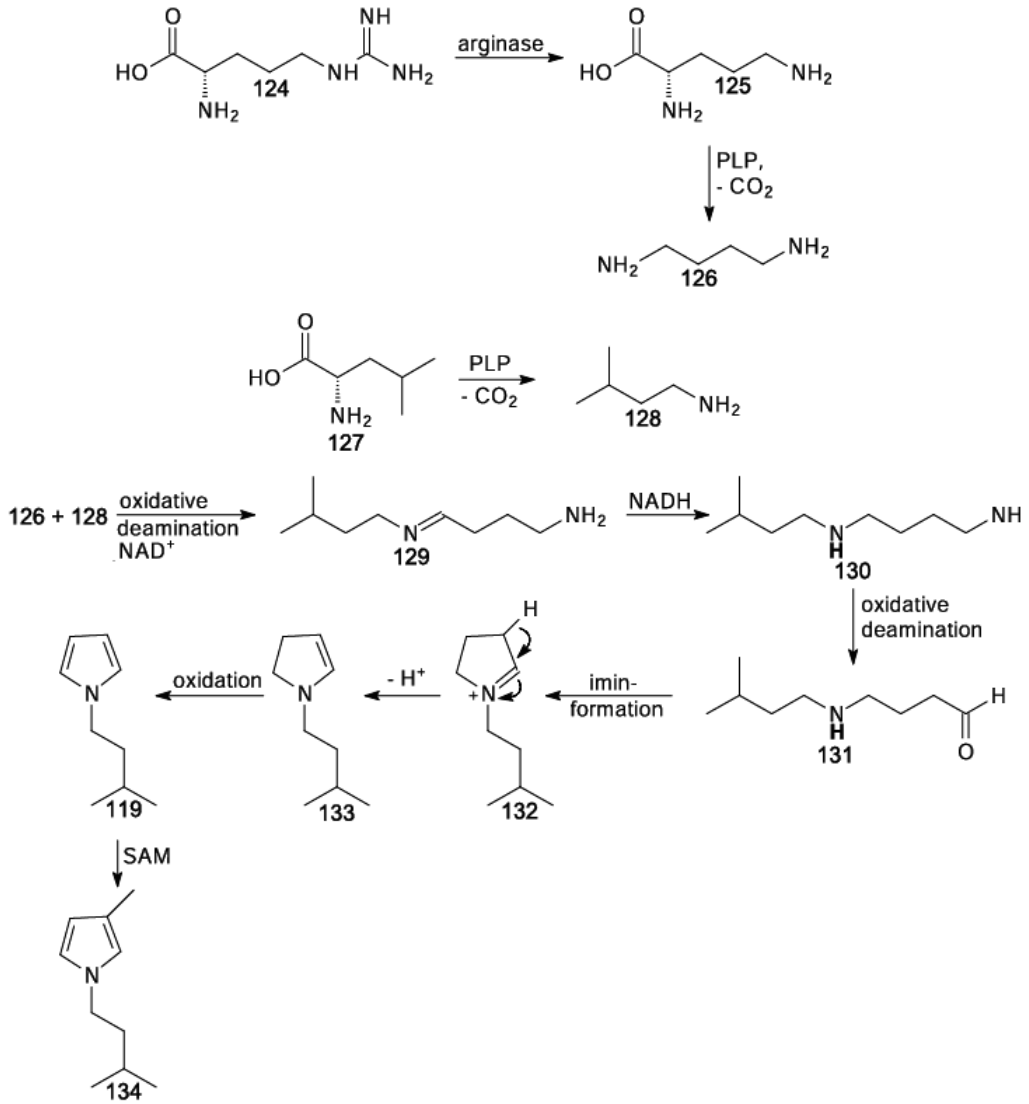

Figure 115: Proposed biosynthesis of *N*-3-methylbutylpyrrole and 3-methyl-*N*-3-methylbutylpyrrole.

L-Arginine **124** and L-leucine **127** could be potential starting compounds in their biosynthesis. The *N*-alkyliminourea moiety of **124** is hydrolyzed to the ω-amino AA **125** followed by a decarboxylation to furnish the diamine **126**. Analogously, AA **127** is converted to the corresponding

amine **128**. Then, the intermediates **126** and **128** are converted in an oxidative deamination to the the imin derivative **129** which is subsequently hydrogenated to the *N*-monoalkylated diamine **130**. Then, **130** is transformed to aldehyde **131** by a second oxidative deamination followed by an intramolecular condensation under formation of the cyclic intermediate **132**. Finally, deprotonation yields **133** which is oxidized to pyrrole **119**. Finally, methylation in 3-position by alkylation with SAM takes place to provide **134** because this position is reactive to electrophilic intermediates as SAM.

Geranylacetone represented a further main component in these experiments. This derivative was beside 10:al the most abundant compound. Geranylacetone was proposed as structure by comparison with a reference spectrum. Furthermore, the *RI* of 1457 was very similar to the recently published value of 1456 [Dickschat *et al.*, 2005b]. The intensity of this compound increased in intensity compared to 10:al in the male/female experiment and the male/female web experiment. This compound was also present in male and female headspace extracts. This compound was recently identified as part of the larval aggregation pheromone of the codling moth *Cydia pomonella* [Jumean *et al.*, 2005]. Several other reports describe the role of geranylacetone in the semiochemistry of insects [pherobase].

Sulcatone, 6-methyl-hept-5-en-2-one, was another important compound in these analyses because it appeared most abundantly when mature male and virgin female encountered. Furthermore, this compound occurred also in the trials with the male and when a male was confronted with the web of a virgin female. In contrast, it played just a minor role in the headspace extracts of virgin females. Earlier investigations demonstrated that sulcatone is formed by an oxidative degradation of the internal double bond of a monoterpene precursor as citral [Kim and Toia, 1989]. This compound occurs widespreadly in flora and fauna and ants are well-known producers [Cavill *et al.*, 1956; Cavill and Hinterberger, 1960]. Furthermore, the hunting spider *Habronestes bradleyi* (Araneae:Zodariidae) locates its prey, *Iridomyrmex purpureus* meat ants, by their alarm pheromone sulcatone [Allan *et al*., 1996].

The terpene-derived 4-methyl-4-ethinyl-γ-butyrolactone (lavender lactone), appearing in relative amounts <1%, was identified in the male experiment and also in the male/female experiment but was absent in the female and male/female web experiment. This compound was characterized by an *RI* of 1056 and its mass spectrum is illustrated in Figure 116. This mass spectrum was identical to a reference spectrum of lavender lactone. This lactone was described as constituent of the essential oil of several plants, for example hops and based on its name also in lavender [Naya and Kotake, 1968; Jin, 2005].

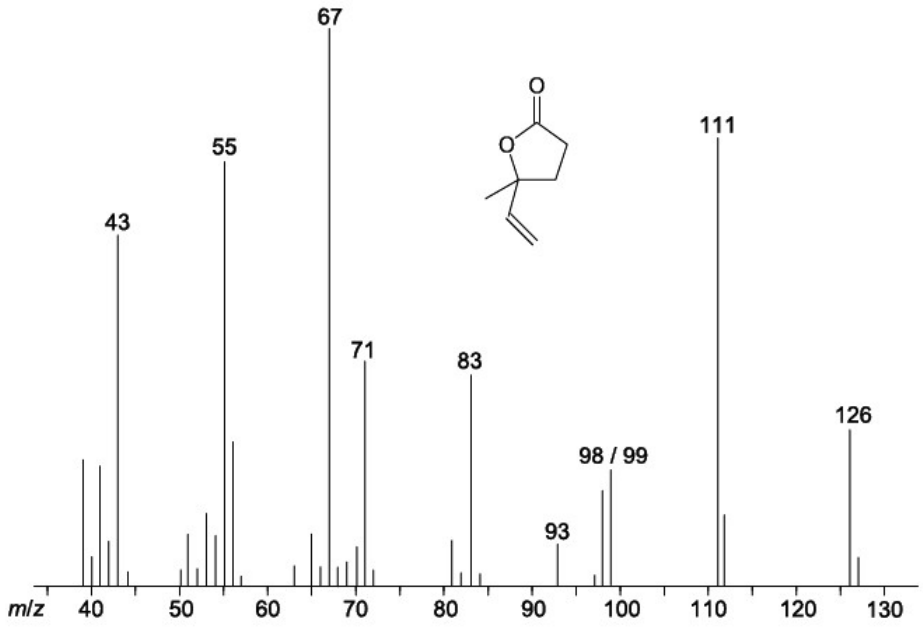

Figure 116: Mass spectrum of 4-methyl-4-ethinyl-γ-butyrolactone (lavender lactone).

This compound is biosynthetically derived from linalool **135** and its formation was proposed according to Figure 117 [Madyastha *et al.*, 1977]. Initially, autoxidation of **135** furnishes the hydroxy-aldehyde **136**, followed by oxidation to the corresponding acid **137**. Final cyclisation provides then lavender lactone **138**.

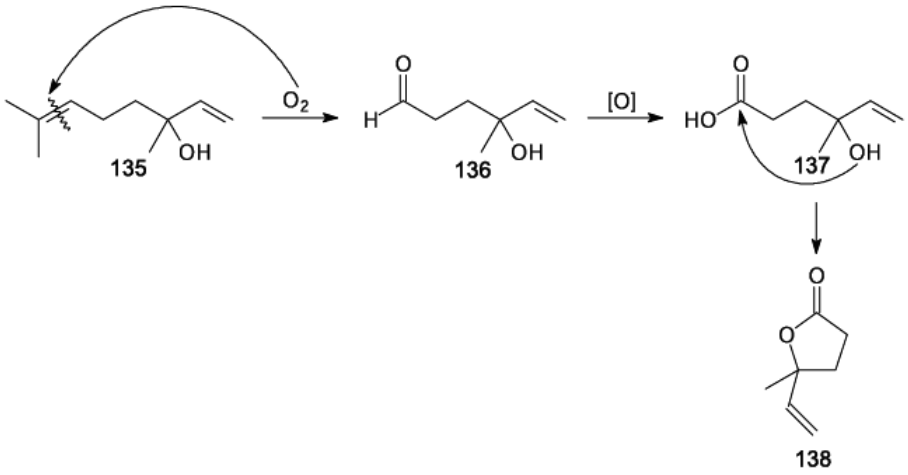

Figure 117: Proposed biosynthetic pathway for the formation of the lavender lactone.

2-Ketones represented another compound class in these experiments occurring as minor compounds. They were not formed by females, except of tridecan-2-one (13:2one), which was

preferentially present in the female headspace extract. The male blend of ketones comprised derivatives in the range of 7 to 16 carbon atoms. Males alone produced these compounds only in traces and they were mainly present in higher amounts in the male/female headspace experiment. Their mass spectra were characterized by the base peak *m*/*z* = 43 and the McLafferty ion *m*/*z* = 58. An increment of 200 points was found for the 2-keto moiety on a BPX-5-phase [J. S. Dickschat, personal communication]. The *RI*s of the natural compounds showed the methyl ketones to be unbranched. Methyl ketones were identified in several species of insects as semiochemicals [pherobase]. Furthermore, 12:2one is a repellent for ticks and maize weevils and it is part of the essential oil of the shrub *Cleome monophylla* [Ndungu *et al.*, 1995].

Unsaturated aldehydes with an (*E*)-configured double bond in 2-position ranging between 9 and 13 carbon atoms were also identifed in the headspace extracts. These compounds were insignificant in the female headspace and occurred mainly in the male/female experiment. The most important derivative was (*E*)-2-dodecenal (2*E*-12:al). A *RI* of 1479 was determined for this compound. An increment of 275 points was found for the (*E*)-configured α,β-unsaturated aldehydes as shown in Table 18. Based on this increment, the (*E*)-configuration for this and other derivatives was proved. The corresponding (*Z*)-isomers elute slightly earlier than the (*E*)-isomers.

Table 18: Retention indices (*RI*s) for (2*E*)-aldehydes on a BPX-5-phase.

Compounds	*RI*
(2*E*)-8:al	1073
(2*E*)-9:al	1175
(2*E*)-10:al	1277

γ-Butyrolactone, δ-valerolactone and their derivatives were identified by comparison with reference mass spectra [NIST database]. These compounds were absent in the female headspace. They occurred in the male/female experiment in concentrations <1%, but they were also present in traces in the male headspace. γ-Butyrolactone, δ-valerolactone and the corresponding 4-alkyl and 5-alkyl derivatives with an even number of carbon atoms were predominant in this blend. A *RI* of 938 was determined for γ-butyrolactone. Earlier, this compound was described as male pheromone in the stink bug *Aethus indicus* (Homoptera) [Olagbemiro *et al.*, 1984]. The corresponding mass spectra of 4-alkylated γ-butyrolactones, as shown for 4-hexyl-γ-butyrolactone in Figure 118, were characterized by the ion pair *m*/*z* = 85 and 100.

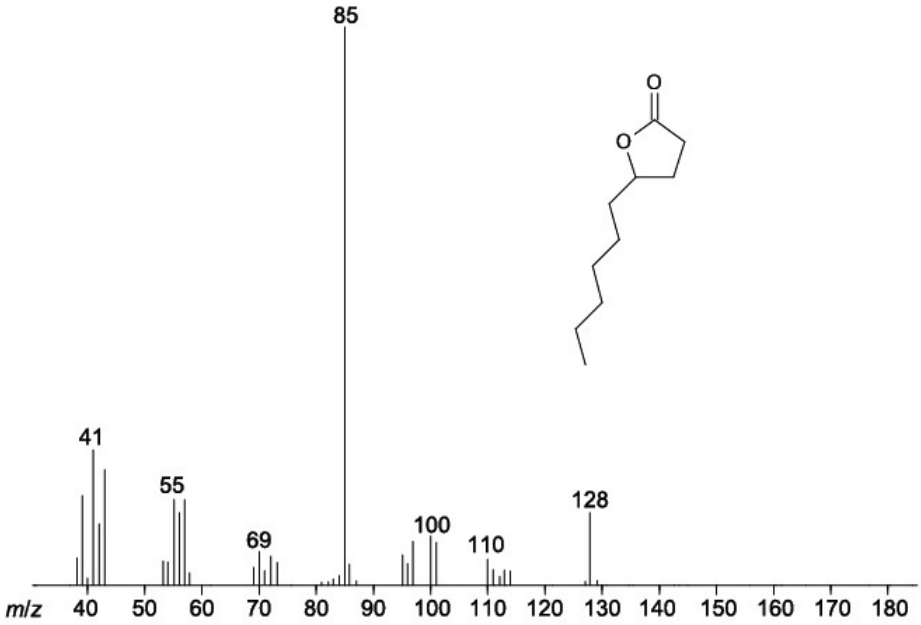

Figure 118: Mass spectrum of 4-hexyl-γ-butyrolactone.

The first one was formed by an α-cleavage of the alkyl chain and the latter one was generated by a McLafferty rearrangement. Furthermore, the ion *m*/*z* = 128 was prominent in the derivative with the hexyl side chain and also in other alkylated γ-butyrolactones with at least 5 carbon atoms in the alkyl chain. Compared to reference mass spectra, a weak loss of water was expected but this ion declined by the noticeable background noise. Based on the general fragmentation pattern, other alkylated γ-butyrolactones were identified and 4-ethyl-γ-butyrolactone and 4-butyl-γ-butyrolactone were the most important derivatives. Analogously, δ-valerolactone and its derivatives were identified. Instead of the ions *m*/*z* = 85 and 100, the ions *m*/*z* = 99 and 114 occurred in the mass spectra of the corresponding 5-alkylated δ-valerolactone. The derivative with the hexyl side chain appeared as most abundant derivative of this compound class.

5.3 A new female spider pheromone of the wasp spider *Argiope bruennichi*

5.3.1 Preface

The wasp spider *Argiope bruennichi*, a female is shown in Figure 119, belongs to the family of araneidae and is closely related to the garden spider *Araneus diadematus* [Wikipedia]. Adult females have a body length of 25 mm in contrast to males with a body length of 6 mm. The web of the wasp spider is characterized by a stabiliment. The function of this stabiliment is still unknown

but it was proposed that it is essential for camouflage. Wasp spiders start to vibrate in their webs if they are in danger and caused by the stabiliment they become unvisible in their webs. Earlier, this species was mainly distributed in the Mediterranean area but occurs also now in the temperate zone, triggered by climate changes.

Figure 119: Female wasp spider *Argiope bruennichi* in her web [Wikipedia].

Earlier, the presence of a volatile female pheromone was described in the closely related species *A. trifolium* [Olive, 1982]. During this thesis, a chemical description of the lipid blend of male and female cuticular, the female web, and the female headspace was performed to investigate if females of this species also produce a pheromone.

5.3.2 Analysis of body extracts of *Argiope bruennichi* females

Body extracts of subadult, virgin, and mated *A. bruennichi* females in dichloromethane were investigated by GC-MS followed by identification of the constituents of these extracts. If necessary, extracts were derivatized to facilitate structure elucidation. The results of these investigations are compiled in Table 19. Figure 120 shows the corresponding chromatograms.

Table 19: Compilation of the chemical composition of body extracts of subadult, virgin, and mated females of *Argiope bruennichi*. Intensities of compounds are given in %.

RI	Compound	Subadult	Virgin	Mated
1518	Trimethyl methylcitrate	-	0.2	-
1809	*O*-octanoyl-β-hydroxybutyrolacton	Tr	0.1	Tr
1994	14:amide	0.4	0.3	0.14
2060	13Me-14:amide	0.1	-	-
2069	12Me-14:amide	0.1	-	-
2099	15:amide	0.2	-	-

RI	Compound	Subadult	Virgin	Mated
2102	9*Z*,12*Z*-18:MeEster	-	1.6	0.1
2106	9*Z*-18:MeEster	0.2	0.7	-
2135	18:MeEster	-	0.3	-
2156	9*Z*,12*Z*-18:Acid	4.3	1.6	3.6
2185	9*Z*-16:amide	0.9	0.4	0.6
2189	9*E*-16:amide	1.0	1.0	0.6
2205	16:amide	1.2	1.1	0.5
2269	15Me-16:amide	0.1	0.3	-
2278	14Me-16:amide	0.2	-	-
2284	9*Z*-17:amide	0.2	-	-
2289	9*E*-17:amide	0.2	-	-
2291	16:*N*,*N*-dimethylethylester	-	0.5	-
2300	23:H	0.1	1.4	0.6
2327	9*Z*,12*Z*-18:hydroxyethylamide	-	0.5	0.2
2339	9*Z*-18:hydroxyethylamide	-	-	0.1
2366	18:hydroxyethylamide	0.1	0.3	0.1
2377	3Me-23:H	-	-	0.1
2391	9*Z*-18:amide	9.1	1.7	1.3
2398	9*E*-18:amide	10.3	5.0	4.6
2400	24:H	-	0.5	0.3
2415	18:amide	0.5	0.3	0.2
2417	18:acetate	-	0.9	0.2
2463	9*Z*,12*Z*-18:*N*,*N*-dimethylethylester	-	1.0	Tr
2468	9*Z*-18:*N*,*N*-dimethylethylester	-	0.2	-
2468	2Me-24:H	0.1	-	0.2
2492	*(Z)*ene-19:amide	Tr	-	-
2494	18:*N*,*N*-dimethylethylester	-	0.3	0.1
2499	*(E)*ene-19:amide	0.1	-	-
2500	25:H	0.9	1.3	1.0
2538	9/11/13-Me-25:H	0.2	0.3	0.3
2541	7Me-25:H	-	0.1	0.1
2573	3Me-25:H	-	0.2	0.1
2598	Xene-20:amide	0.1	-	-
2600	26:H	0.5	1.0	0.5
2619	20:acetate	-	0.8	0.3
2627	X,X,X,Xtetraene-20:*N*,*N*-dimethylethylester	-	0.2	-
2634	9/10/11/12/13Me-26:H	-	0.3	-
2642	6Me-26:H	-	0.2	-
2668	2Me-26:H	0.6	0.9	0.6
2700	27:H	1.0	1.4	1.2
2738	9/11/13Me-27:H	0.5	0.7	0.4
2746	7Me-27:H	0.2	0.3	0.1
2769	2Me-27:H	0.3	0.4	0.4

RI	Compound	Subadult	Virgin	Mated
2779	3Me-27:H	0.4	0.5	1.0
2800	28:H	-	1.3	1.2
2809	Xene-22:amide	1.6	-	-
2838	12/13/14Me-28:H; +9/10/11 virg.	0.6	0.9	0.5
2843	6Me-28:H	-	0.2	-
2871	2Me-28:H	5.0	5.7	5.2
2900	29:H	1.6	1.5	2.0
2918	Cholesta-3,5-diene	0.2	0.3	0.2
2939	9/11/13Me-29:H	2.5	5.3	3.2
2947	7Me-29:H	0.5	0.6	0.5
2955	5Me-29:H	0.2	0.2	0.2
2964	9,13-DiMe-29:H	0.3	0.3	0.4
2970	2Me-29:H	0.5	0.5	0.5
2981	3Me-29:H	0.6	0.5	1.2
3000	30:H	0.6	0.4	0.9
3038	9/10/11/12/13/14/15Me-30:H	1.5	2.5	1.7
3052	6Me-30:H	-	-	0.1
3070	2Me-30:H	2.9	2.7	3.6
3100	31:H	0.7	0.3	0.9
3137	9/11/13/15Me-31:H	3.8	6.4	4.5
3159	Cholesterol	16.4	16.3	28.5
3181	3Me-31:H	0.5	0.7	Tr
3200	32:H	0.8	0.4	1.1
3235	10/11/12/13/14/15/16Me-32:H	1.2	1.7	1.5
3249	Ergosterol	0.4	-	0.3
3261	13,17-DiMe-32:H	0.7	0.6	0.8
3275	2,(4/6/8)DiMe18Ac:13OH	0.9	0.5	0.9
3287	Cholesterone	0.3	0.2	0.4
3338	11/13/15/17Me-33:H	2.8	3.6	2.7
3357	13,19/13,17-DiMe-33:H	1.7	1.8	2.0
3379	2,(4/6/8)DiMe19Ac:13OH	4.8	3.1	2.6
3449	12/13/14/15/16/17Me-34:H	0.6	0.6	0.7
3475	15,17/15,19/13,21-DiMe-34:H	0.7	0.7	0.7
3501	2,4DiMe19Ac:14OH; 2,4DiMe20Ac:13OH	2.7	1.3	1.5
	11/13/15/17Me-35:H	0.6	0.9	0.6
	15,17-DiMe-35:H	1.1	1.6	0.9
	2,4DiMe21Ac:13OH	1.5	1.0	0.9

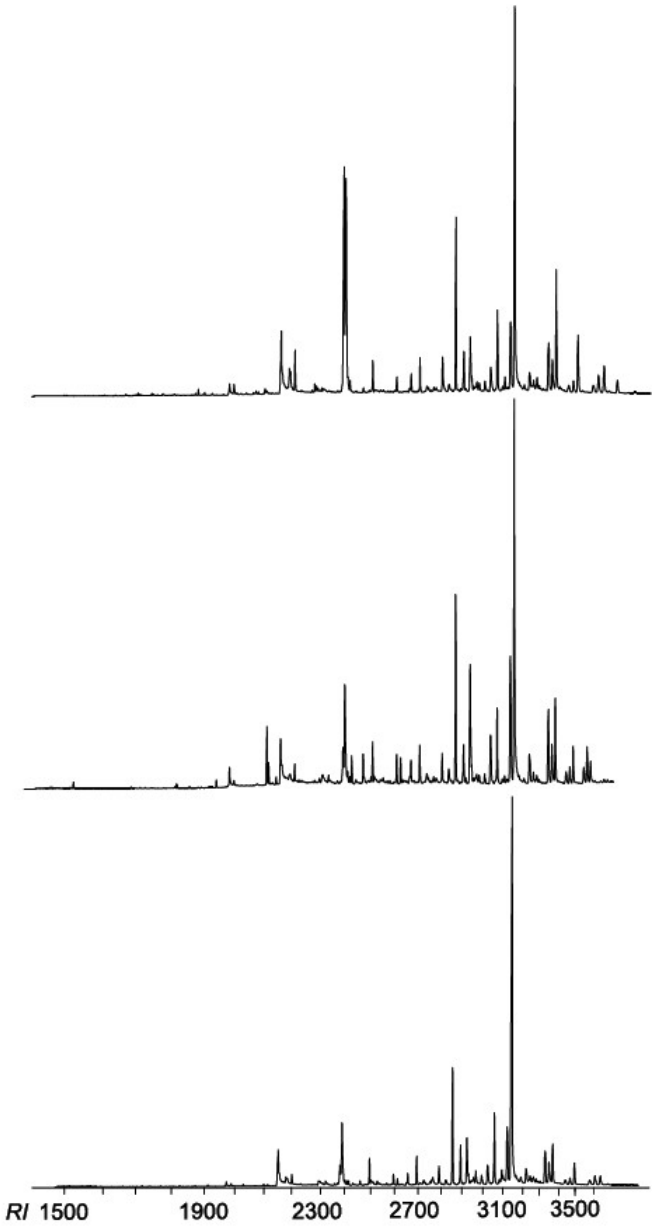

Figure 120: Total ion chromatograms of body extracts of *Argiope bruennichi* females. Top: subadult; middle: virgin; bottom: mated.

Different lipid classes as alkanes, amides, and wax esters occurred in these extracts. The blend of fatty acid amides (FAAs) comprised unsaturated amides as well as unbranched and branched saturated derivatives. Wax esters consisted of branched acids and unbranched alcohols and the alkane blend contained unbranched, monomethyl and dimethyl branched derivatives.

Unsaturated FAAs were identified in the extracts independent of the developmental stage but they were more prominent in extracts of subadult females than in the other ones. The branched and unbranched saturated FAAs were mainly present in the body extract of the subadult females and

branched amides with methyl branches in (ω-1) or (ω-2)-position were present. All investigated subadult body extracts contained a 1:1-ratio of (*Z*)-9-octadecenamide (9*Z*-18:amide) and the corresponding (9*E*)-isomer whereas this mixture changed to a *Z*/*E*-ratio of 1:5 in virgin and mated females. These compounds contributed ca. 20% to the TIC in subadult females in contrast to a contribution of ca. only 6% in virgin and mated female body extracts. The mass spectrum of (9*Z*)-octadecenamide is illustrated in Figure 121.

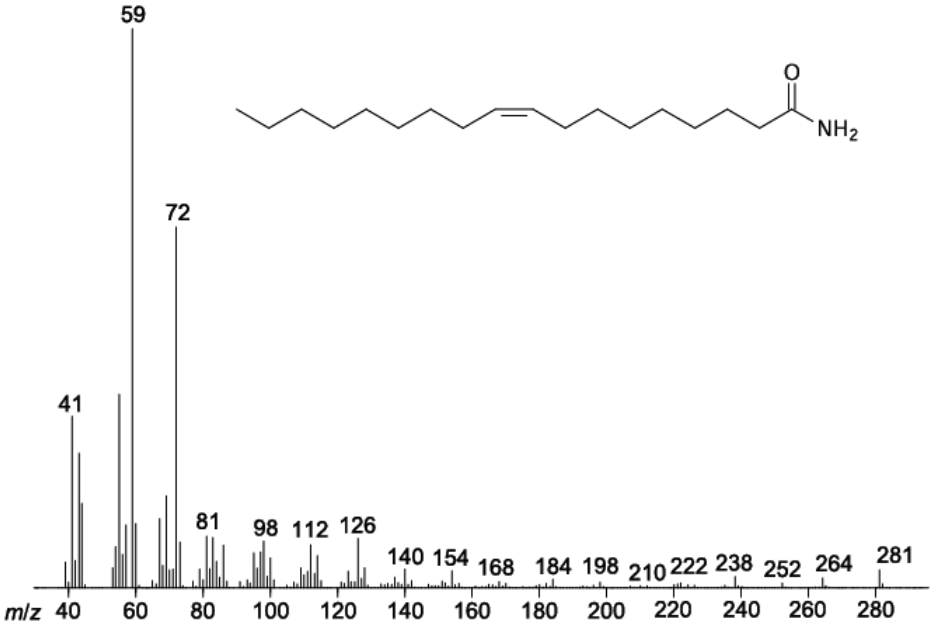

Figure 121: Mass spectrum of (*Z*)-9-octadecenamide.

The mass spectrum was characterized by the ion pair *m*/*z* = 59 and 72 indicating the presence of an amide. These ions were formed by a β-cleavage and a McLafferty rearrangement. The unsaturated amides ranged from 16 to 22 carbons and therein 9*Z*-, 9*E*-16:amide, and 13-22:amide were additionally important. Double bond positions were partly elucidated by analysis of the mass spectra of the corresponding DMDS-adducts as shown in Figure 122 for 9*Z*-18:amide. The ions *m*/*z* = 173 and 202 were formed by the C-C-cleavage between the vicinal thiomethyl groups and loss of ammonia from the fragment *m*/*z* = 202 provided the ion *m*/*z* = 185. The (*Z*)- and the (*E*)-isomer (**139** and **140**) were synthesized as reference compounds by amination of the corresponding acid chlorides in 54% and 73% yield and their data were identical with those of the natural compounds.
Correspondingly, an unsaturation in position 9 in the hexadecenamides and heptadecenamides was confirmed by the observation of the ions *m*/*z* = 145, 185, and 202 for the hexadecenamides and 159, 185, and 202 for the heptadecenamides. The double bond position in the other unsaturated derivatives remained unclear.

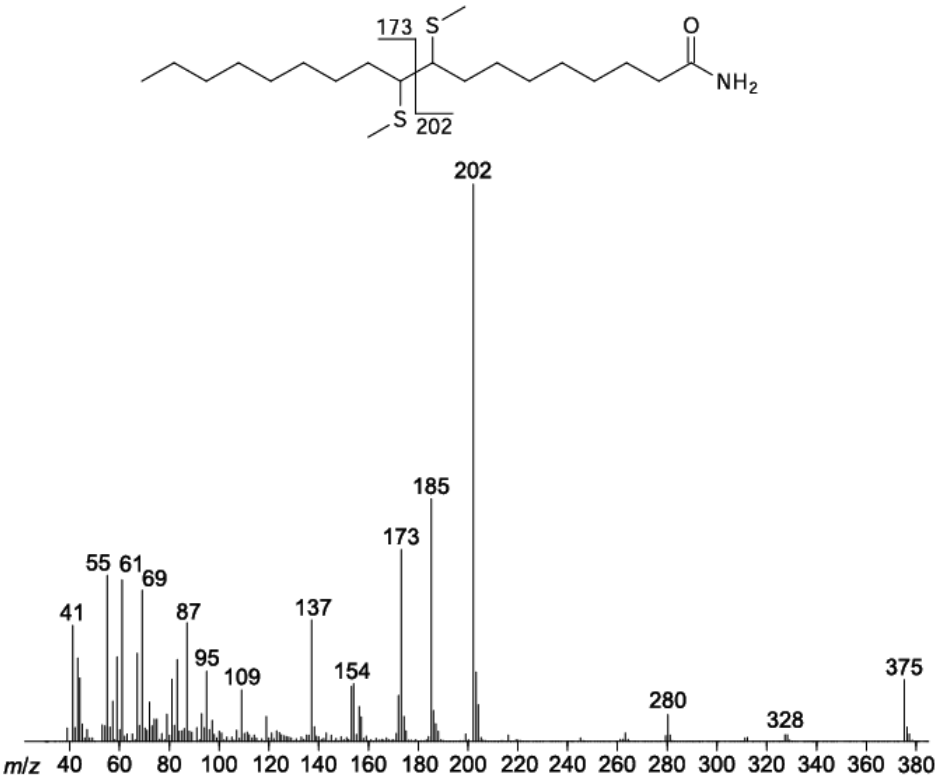

Figure 122: Mass spectrum of the corresponding DMDS-adduct of (*Z*)-9-octadecenamide.

FAAs are signal lipids which are involved in many physiological and pathological processes in mammals [Cravatt and Lichtman, 2002]. Important representants of this lipid class are *N*-arachidonyl ethanolamine and also 9*Z*-18:amide. The latter one is a sleep-inducing lipid and their signal function is putatively terminated by hydrolysis to the corresponding free acid [Cravatt *et al.*, 1995]. In contrast, bioactivity for the (9*E*)-isomer is unknown.

The chemical composition of the virgin and mated body extract was different compared to that of the subadults and especially the blend of amides was only of subordinate importance. Instead of these compounds, *N*,*N*-dimethylaminoethyl esters and *N*-acylaminoethanol (NAEs) derivatives occurred and the corresponding derivatives with linoleic acid represented the most prominent derivatives. These compounds contributed 1.0% and 0.5% to the TIC and their mass spectra are depicted in Figure 123 and 124.

The ion pair *m*/*z* = 58 and 71 was important for the identification of the *N*,*N*-dimethylaminoethylacyl moiety. The first one exhibited the base ion in this mass spectrum and both were formed by common fragmentation pathways of fatty acid derivatives after cleavage of the dimethylaminoethyl moiety followed by hydrogen transfer. The ion pair *m*/*z* = 131 and 144, though appearing in small intensity and not visible in Figure 122, were formed by the same pathway before

this unit was cleaved. The compound had a molecular weight of 351 amu and under assumption of a carbon chain with 18 carbon atoms, the presence of two double bonds was proved. A 9,12-arrangement of the double bond was proposed because linoleic acid represents a common diunsaturated acid with 18 carbon atoms in nature, confirmed by identical mass spectra and *RIs* between natural and synthetic sample. The synthetic sample **141** was accessible after esterification of linoleic acid with *N*,*N*-dimethylaminoethanol in 87 % yield. Earlier, this compound was identified in bovine liver accompanied by other derivatives of this compound class [Jandke and Spiteller, 1988].

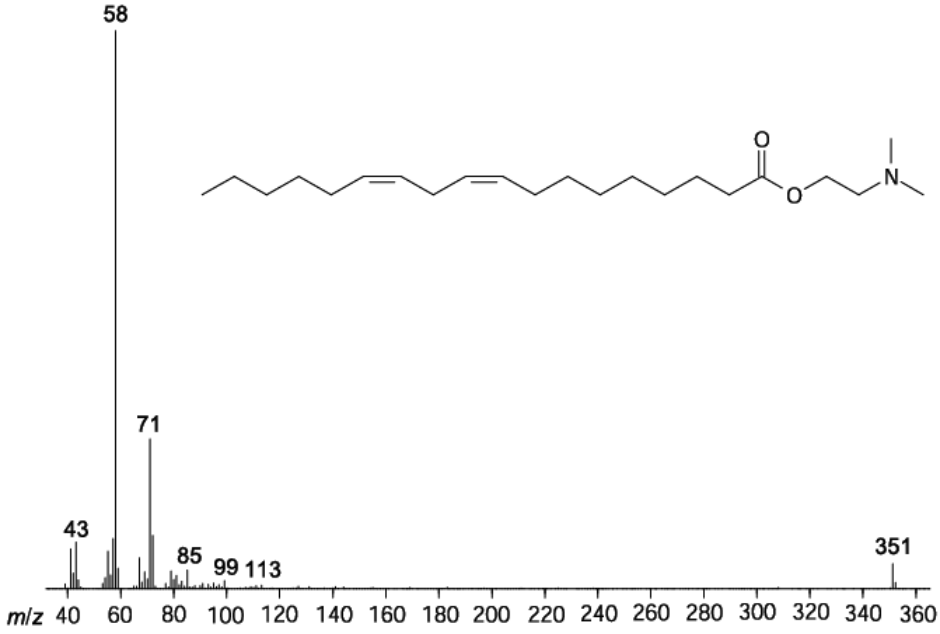

Figure 123: Mass spectrum of *N*,*N*-dimethylaminoethyl α-linoleate.

Mass spectra of NAEs were characterized by the ion *m*/*z* = 85 and the base ion *m*/*z* = 98. These ions were formed by a β-cleavage and a McLafferty rearrangement after loss of water. The ion *m*/*z* = 305 exhibited the molecular ion and a diunsaturated acid with 18 carbon atoms was deduced The ions *m*/*z* = 154, 194, and 248 were enhanced in intensity compared to the surrounding ones and indicated the double bonds to be in position 9 and 12, because of the preferred cleavages in allylic position to the double bonds. This proposal was proved by identical mass spectra and *RI*s between reference and natural compound. Compound **142** was synthesized by amination of the corresponding acid chloride in 93% yield.

Derivatives of this compound class are widespread and involved in the energy intake of vertebrates [Astarita *et al.*, 2005]. For example, oleoylethanolamide (OEA) inhibits feeding in rats and mice and in rodents, the intestinal concentration of OEA increases after refeeding. Analogously, a

300-fold higher level of OEA was observed in the small intestine of fed Burmese python *Python molorus* compared to fasted ones. Plant seeds as pea, cotton, or peanut are also known as sources of NAEs and a role in the regulation of seed germination was proposed for these derivatives [Chapman *et al.*, 1999].

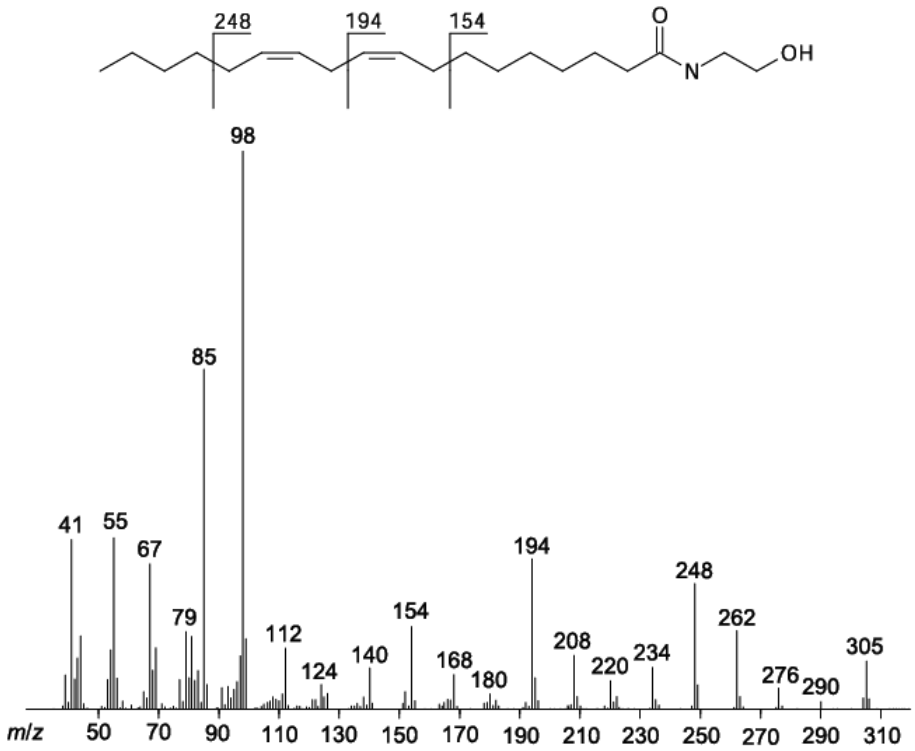

Figure 124: Mass spectrum of α-linoleoyl ethanolamide.

Aminoethanol and *N*,*N*-dimethylaminoethanol are formed from the AA serine by decarboxylation in the presence of PLP as cofactor [Stekol, 1958]. Additionally, the formation of the latter one requires dimethylation by SAM. The dimethylation is similar to the introduction of a protection group in organic synthesis because these biosynthetic steps allow selectively *O*-acylation. In contrast, *N*-acylation is preferred in the absence of the two methyl groups.

Unbranched and branched alkanes in the range of 23 to 35 carbon atoms represented further derivatives in the lipid blend of all developmental stages. Monomethyl branched alkanes with internal and terminal methyl branches as well as dimethyl branched ones with internal methyl groups were identified in these extracts. Mass spectra of wax esters contained the ion m/z = 74 indicating the presence of a methyl group at C-2 in the acid. Analysis of the wax ester composition was difficult caused by coelution of isomeric wax esters differing in the chain length of the alcohol and the acid. Transesterification of the wax esters with TMSH followed by derivatization with sodium pyridylmethanolate demonstrated the existence of a second methyl branch in the acid of the

wax ester occurring mainly in 4-, 6-, or 8-position. The alcohols of these wax esters were derivatized with MSTFA and elucidation of their *RI*s showed the absence of methyl branches. Tridecanol and tetradecanol occurred as alcohols in these derivatives. All extracts were dominated by a blend of 2,(4/6/8)DiMe19Ac:13OH and these isomers were present in amounts of 2.6 – 4.8%. Trimethyl methylcitrate and *O*-octanoyl-β-hydroxybutyrolactone were also identified but these compounds occurred preferentially in body extracts of virgin females.

5.3.3 Chemical composition of web extracts of *Argiope bruennichi* females

Web extracts of virgin and mated female *A. bruennichi* in dichloromethane consisted mainly of *n*-alkanes, monomethyl branched alkanes, dimethyl branched alkanes, and wax esters. Minor compounds as trimethyl methylcitrate and *O*-alkanoyl-β-hydroxybutyrolactones completed these blends. Table 20 summarizes the identified compounds in these extracts and corresponding TICs are depicted in Figure 125.

Unbranched alkanes contributed 37.6% to the TIC of a virgin web extract and their chain length ranged from 16 to 35 carbon atoms. Nonacosane (19:H) represented with 6.2% the most abundant representative. Even-numbered alkanes occurred in similar quantities as the odd-numbered ones and octacosane (18:H) occurred as most abundant representative with an amount of 5.9%. Normally, odd-numbered alkanes prevail in cuticular alkane mixtures of arthropods because acetate is rather used as starter unit than propionate. But here, the distribution of alkanes shows clearly that acetate and propionate were equitably used as starter units in the biosynthesis of alkanes. Monomethyl branched alkanes formed the most abundant compound class in web extracts of virgin females with 39.3% and the chain length ranged from 30 to 35 carbon atoms. The methyl branches in odd-numbered alkanes were attached either at C-2 or C-3 or internal in odd-numbered positions. Even-numbered and odd-numbered dimethyl branched alkanes accounted for 14.3% of the TIC of a virgin web extract and their methyl branches were internally arranged.

Another lipid class was represented by wax esters consisting of a dimethyl branched acid and an unbranched alcohol. These derivatives contributed 5.2% to the TIC. The acids of these wax esters were similar to those of the body extracts. The alcohols were also unbranched and comprised 13, 14, or 15 carbon atoms.

The web extracts of mated females comprised the same lipid classes but their amount differed slightly. Wax esters contributed 15.6% to the TIC and exceeded the dimethyl branched alkanes with 11.5%. Monomethyl branched alkanes represented the most abundant compound class with 38.6% followed by *n*-alkanes with 30.6%.

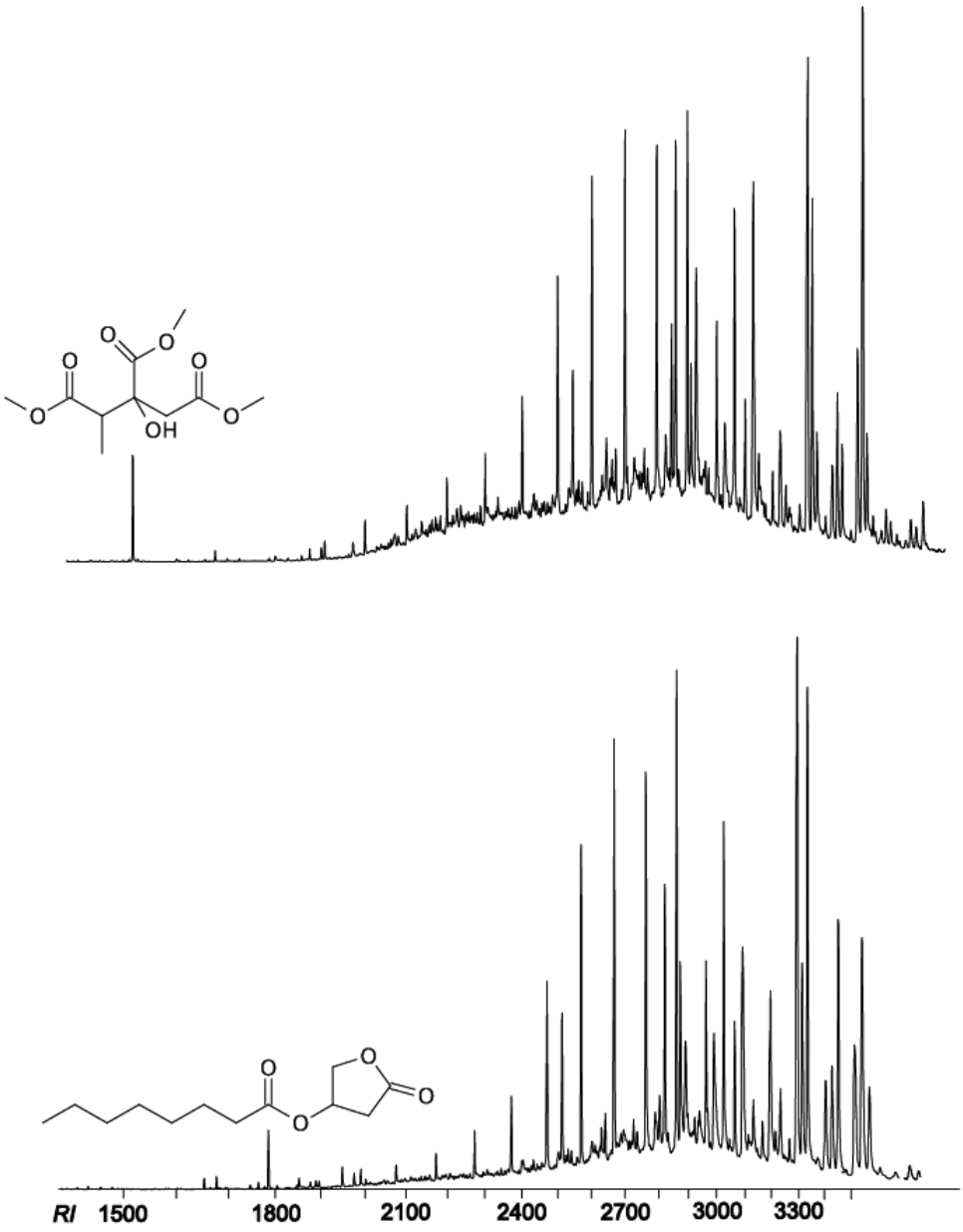

Figure 125: Total ion chromatograms of web extracts of virgin and mated females of *Argiope bruennichi*. Top: virgin; bottom: mated.

Table 20: Composition of web extracts of virgin and mated *Argiope bruennichi* females. Intensities of compounds are given in %.

RI	Compound	Virgin	Mated
1518	Trimethyl methylcitrate	0.6	-
1527	Trimethyl methylcitrate	Tr	-
1600	16:H	Tr	-
1623	14:al	Tr	-
1700	17:H	Tr	-
1800	18:H	Tr	Tr
1809	*O*-octanoyl-β-hydroxybutyrolactone	-	0.5
1827	16:al	Tr	Tr
1900	19:H	0.2	Tr
2000	20:H	0.4	Tr
2015	*O*-decanoyl-β-hydroxybutyrolactone	-	0.1
2100	21:H	0.6	0.3
2200	22:H	1.0	0.3
2300	23:H	1.2	0.8
2400	24:H	2.1	1.0
2500	25:H	3.5	2.3
2600	26:H	4.0	3.2
2700	27:H	4.1	4.0
2762	2Me-27:H	1.2	1.1
2772	3Me-27:H	1.6	1.1
2800	28:H	5.9	4.9
2863	2Me-28:H	4.0	3.5
2872	3Me-28:H	-	1.1
2900	29:H	6.2	6.1
2916	27:al	3.0	3.1
2930	11/13/15Me-29:H	4.8	3.5
2947	5Me-29:H	0.8	-
2961	2Me-29:H	1.2	1.1
2972	3Me-29:H	1.2	0.8
3000	30:H	3.2	2.1
3028	10/11/12/13/14/15Me-30:H	2.6	-
3062	2Me-30:H	2.2	4.6
3100	31:H	2.0	2.4
3130	11/13/15Me-31:H	6.1	4.7
3150	13,17DiMe-31:H	1.2	0.8
3155	9,13DiMe-31:H	0.8	-
3161	2,(4/6/8)DiMe17Ac:13OH	-	1.5
3200	32:H	1.4	1.5
3228	10/11/12/13/14/15/16Me-32:H	2.2	3.7

RI	Compound	Virgin	Mated
3249	13,17DiMe-32:H	1.0	1.1
3261	2,(4/6/8)DiMe18Ac:13OH	-	1.7
3263	2Me-32:H	0.6	-
3300	33:H	0.8	0.8
3331	11/13/15/17Me-33:H	6.4	7.8
3349	13,17/13,19DiMe-33:H	3.6	3.2
3367	2,(4/6/8)DiMe19Ac:13OH	2.2	6.3
3400	34:H	0.6	0.9
3427	13/14/15/16/17Me-34:H	1.6	2.3
3447	13,19/15,21DiMe-34:H	1.8	2.0
3465	2,(4/6/8)DiMe19Ac:14OH	1.6	4.0
3500	35:H	0.4	-
3527	11/13/15/17Me-35:H	2.8	3.3
3547	13,19/13,21DiMe-35:H	5.9	4.4
	2,(4/6/8)DiMe19Ac:15OH	1.4	2.1

Methyl trimethylcitrate **146** (Figure 126) was present in virgin web extracts and accounted for 0.6% of the TIC. On the other hand, this compound was absent in most investigated mated web extracts. This compound is closely related to the female sex pheromone, (*S*)-dimethyl citrate, of the ctenid spider *Cupiennius salei* [Tichy *et al*., 2001]. Furthermore, its outstanding presence in all investigated virgin web extracts and absence in the mated web extracts were interesting.

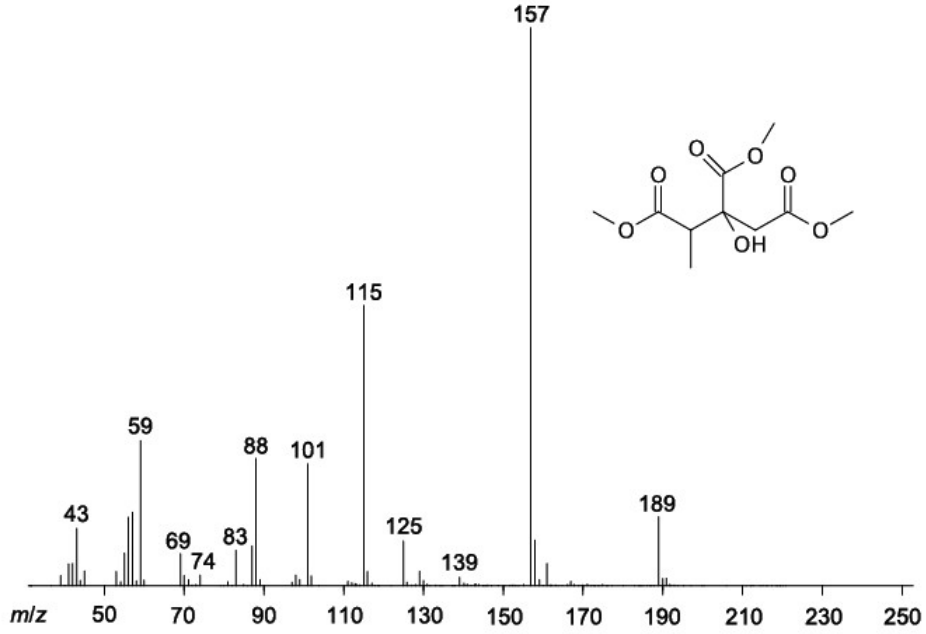

Figure 126: Mass spectrum of trimethyl methylcitrate.

An ion representing the molecular ion was not visible in the mass spectrum of **146**. The ion *m*/*z* = 189 was formed by a α-cleavge of the methoxycarbonyl unit at the quarternary carbon atom. An elimination of methanol provided the base ion *m*/*z* = 157. Furthermore, elimination of methyl acetate (74 amu) and methyl propionate (88 amu) from the ion *m*/*z* = 189 furnished the ions *m*/*z* = 101 and 115.

Derivatization with MSTFA converted this compound into a derivative with a molecular weight of 320 amu, indicated by the ion *m*/*z* = 305 (Figure 127).

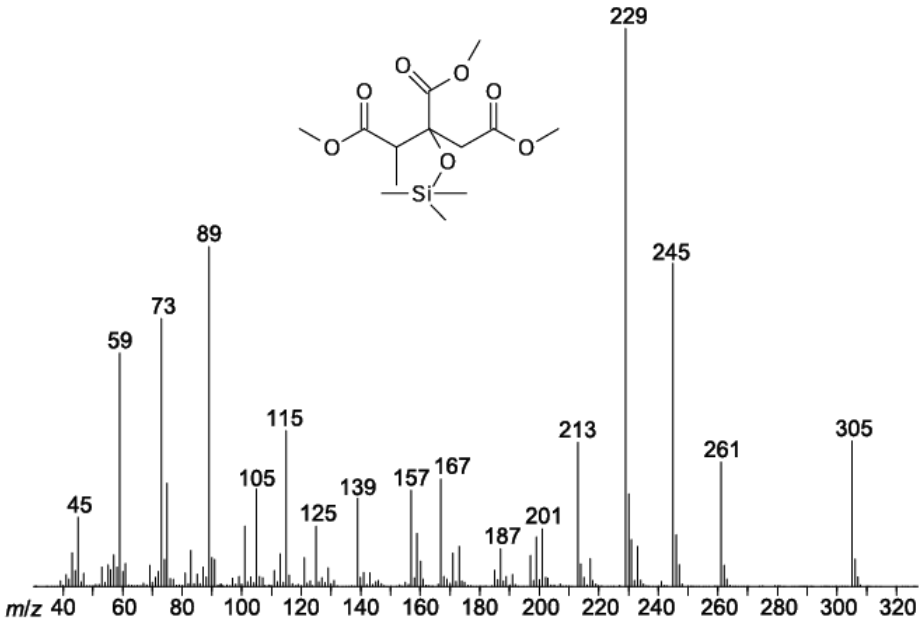

Figure 127: Mass spectrum of the corresponding trimethylsilyl ether of trimethyl methylcitrate.

This result was explainable by the uptake of one TMS-group leading to a shift from the ion *m*/*z* = 248 to the ion *m*/*z* = 320. Compound **146** occurred in a diastereomeric ratio of 25:1 (in average) in virgin web extracts and the major diastereoisomer was characterized by a *RI* of 1518 and the minor one by a *RI* of 1527.

The structural proposal based on mass spectral interpretation was confirmed by synthesis of racemic **148** according to Figure 128.

Racemic malic acid **143** was transformed into compound **144** by treatment with 2,2-dimethylpropanal in 60% yield. Alkylation of **144** with methyl 2-bromopropionate followed by methylation with BF_3 in methanol provided the protected trimethyl methylcitrate **145** only in traces in a 1:1-mixture with a coupling product of methyl 2-bromopropionate with itself. Nevertheless, **145** was deprotected to compound **146** by stirring with sodium hydrogencarbonate in methanol. Mass spectral data as well as the *RI*s of the obtained diastereoisomers were identical to those of the

natural diastereomeric mixture of **146**.

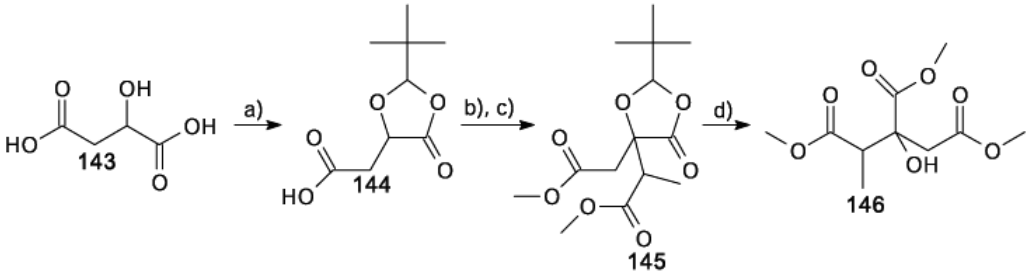

Figure 128: Synthesis of racemic trimethyl methylcitrate. a) *p*-TsOH, H_2SO_4, pentane, 2,2-dimethylpropanal, reflux 8h, 60%; b) LiHMDS, methyl 2-bromopropionate, 2h -78°C; c) BF_3, MeOH, rt overnight; d) $NaHCO_3$, MeOH, 24h rt.

Two acylated *β*-hydroxybutyrolactones were present. Often, these lactones occurred exclusively in web extracts of mated females and the *β*-hydroxybutyrolactone core was acylated with octanoic acid (0.5% of the TIC) or decanoic acid (0.1% of the TIC). Acylated *β*-hydroxybutyrolactones were unknown before as natural compounds. The mass spectrum of the major derivative, *O*-octanoyl-*β*-hydroxybutyrolactone **147**, is illustrated in Figure 129.

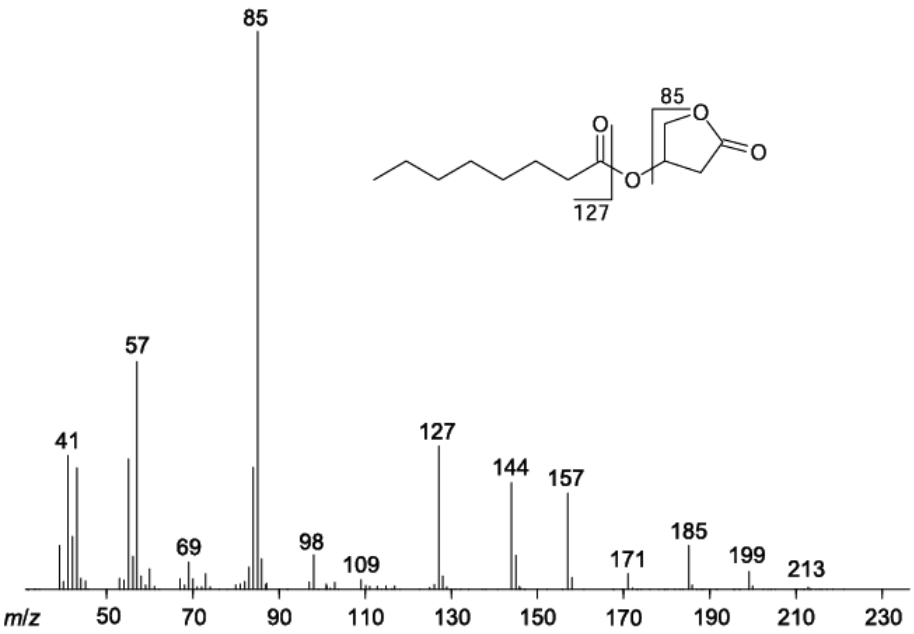

Figure 129: Mass spectrum of (*S*)-*O*-octanoyl-*β*-hydroxybutyrolactone.

The base ion *m*/*z* = 85 is formed by a cleavage of the butyrolactone ring. A α-cleavage under release of an octanoyl moiety furnishes the ion *m*/*z* = 127. A McLafferty rearrangement under elimination of hexene provides the ion *m*/*z* = 144, analogously to other fatty acid derivatives. The derivative was

characterized by a *RI* of 1809 and a synthetic reference of **147** showed a identical *RI* and mass spectral data. The synthetic reference of **147** was accessible after esterification of (*S*)-*β*-hydroxybutyrolactone with octanoic acid in 72% yield.

Putatively, these compounds are biosynthetically derived by malic acid according to Figure 130. Malic acid **143** is regioselectively reduced under formation of 3,4-dihydroxybutanoic acid **148**, followed by cyclisation to the *β*-hydroxybutyrolactone core **149**. Then, final acylation provides the derivatives **150**.

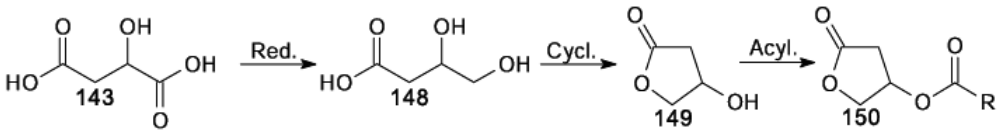

Figure 130: Proposed biosynthesis of *O*-acyl-*β*-hydroxybutyrolactones.

5.3.4 Chemical composition of body extracts of *Argiope bruennichi* males

Body extracts of virgin and mated males in dichloromethane were investigated by GC-MS. Table 21 compiles the identified compounds of these extracts and Figure 131 shows the corresponding TICs. The body extracts of virgin *A.* b*ruennichi* males consisted of amides, alkanes, methoxyalkanes and wax esters. The TIC of virgin males was characterized by a 1.4:1.0-ratio of 9*E*-18:amide and 9*Z*-18:amide, and those represented the most abundant amides in these extracts. This ratio increased in favour of the 9*E*-isomer to a 1.9:1.0-ratio in mated males; a tendency also observed in body extracts of virgin and mated females. The blend of amides accounted for 31.1% of the TIC of virgin males and for 25.9% in the corresponding extract of mated males. Furthermore, the mixtures comprised *n*-alkyl amides, branched amides, and unsaturated amides.

Furthermore, 1-methoxyalkanes and wax esters completed the composition of the body extracts. Methoxyalkanes were male-specific and these derivatives were identified by comparison of mass spectra and *RI*s with those of reference compounds. Their chain length ranged from 22 to 28 carbon atoms and these derivatives contributed 24.5% to an extract of virgins and 27.8% to an extract of mated males. The mass spectrum of 1-methoxyhexacosane (26:1-OMe), representing the base peak of both extracts, is illustrated in Figure 132. The presence of the ion m/z = 45 and the unreactivity of the compound against MSTFA demonstrated the existence of the 1-methoxy unit. The ion pair m/z = 336 and 364 indicated indirectly the molecular weight of 396 amu indicative for 26 carbon atoms. The *RI* of 2834 corresponded to the theoretical value of unbranched 26:1-OMe.

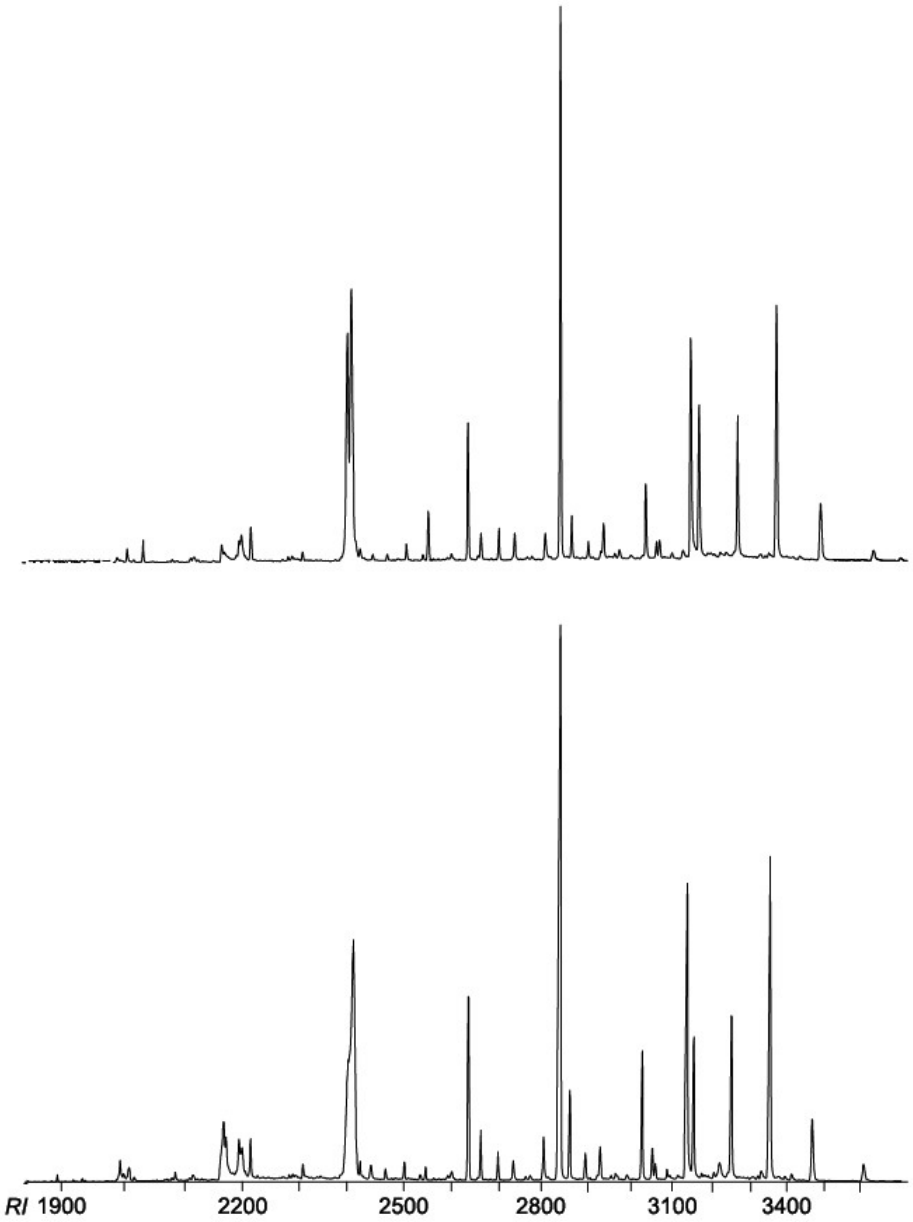

Figure 131: Total ion chromatograms of body extracts of virgin and mated males of *Argiope bruennichi*. Top: virgin; bottom: mated.

Wax esters, consisting mainly of dimethyl branched acids and *n*-alcohols, occurred in virgin and mated body extracts and these derivatives accounted for 24.1% and 22.2% of the TIC. Acids with 2,6-, 2,8-, and 2,12-arrangement of the methyl groups were identified after transformation of the methyl esters into pyridylmethyl esters. The absence of methyl groups in the alcohol moieties of wax esters was confirmed by the mass spectra of the corresponding nicotinic ester derivatives. The blend of wax esters comprised mainly dimethylated acids with a chain length between 17 and 19 carbon atoms and *n*-alcohols with 12 to 14 carbon atoms. Structures of minor wax esters were not

elucidated because of the low recovery of the corresponding pyridylmethyl and nicotinic acid esters.

Table 21: Compilation of the chemical composition of body extracts of virgin and mated *Argiope bruennichi* males. Intensities of compounds are given in %.

RI	Compound	Virgin	Mated
1994	14:amide	0.5	0.4
2060	13Me-14:amide	0.1	0.1
2069	12Me-14:amide	0.1	0.1
2139	18:MeEster	0.1	0.1
2153	9*Z*,12*Z*-18:COOH	0.6	2.7
2158	9*Z*-18:COOH	0.6	1.7
2185	18:COOH	-	1.5
2185	9*Z*-16:amide	0.7	1.2
2189	9*E*-16:amide	1.2	0.4
2205	16:amide	1.2	1.3
2216	18:OAc	-	0.2
2278	14Me-16:amide	0.2	0.2
2284	9*Z*-17:amide	0.2	-
2289	9*E*-17:amide	0.1	-
2300	23:H	0.3	0.5
2327	9*Z*,12*Z*-18:ethanolamide	-	0.2
2339	9*Z*-18:ethanolamide	-	0.2
2391	9*Z*-18:amide	10.3	7.1
2398	9*E*-18:amide	14.7	13.2
2415	18:amide	0.6	0.5
2437	22:1-OMe	0.5	-
2462	2Me-24:H	0.2	0.4
2475	Xene-25:H	0.1	0.1
2482	Xene-25:H	0.2	0.2
2500	25:H	0.7	0.5
2539	23:1-OMe	0.3	0.2
2562	2Me-25:H	0.1	0.1
2572	3Me-25:H	0.1	0.1
2598	Xene-20:amide	0.2	0.2
2600	26:H	0.5	0.5
2618	22:OAc	0.1	0.1
2635	24:1-OMe	3.7	4.0
2663	2Me-26:H	0.9	1.1
2700	27:H	1.0	0.7
2735	25:1-OMe	1.0	0.6

RI	Compound	Virgin	Mated
2762	2Me-27:H	0.3	0.2
2772	3Me-27:H	0.2	0.2
2809	Xene-22:amide	1.0	1.2
2834	26:1-OMe	15.4	19.0
2863	2Me-28:H	1.5	2.0
2900	29:H	0.9	0.7
2929	11/13/15Me-29:H	0.3	0.2
2962	9,19DiMe-29:H; 2Me-29:H	0.4	0.6
2973	3Me-29:H	0.6	0.5
3000	30:H	0.3	0.5
3004	3,9DiMe-29:H	0.2	-
3028	11/12/13/14/15Me-30:H	0.2	0.2
3035	28:1-OMe	0.9	1.2
3059	2Me-30:H	1.4	1.9
3069	2,(6/8/12)DiMe17Ac:12OH	1.2	1.2
3100	31:H	0.3	0.4
3128	11/13/15Me-31:H	0.4	0.6
3138	7/9Me-31:H	0.1	0.2
3151	11,15/13,17DiMe-31:H	0.2	0.3
3169	2,(6/8/12)DiMe17Ac:13OH	11.2	12.2
3189	Ac20:13OH	0.2	0.5
3199	Ac20:13OH	0.2	0.3
3207	Ac19:14OH	0.2	0.4
3221	Ac20:13OH	0.4	0.6
3265	2,(6/8/12)DiMe18Ac:13OH	7.7	9.8
3291	Ac20:14OH	0.2	0.3
3312	Ac21:13OH	-	0.2
3321	Ac20:14OH	-	0.1
3327	11/13/15Me-33:H	0.2	0.3
3339	Ac21:13OH	0.1	0.3
3352	13,17/13,19DiMe-33:H	-	0.3
3369	2,(6/8/12)DiMe19Ac:13OH	14.4	18.9
3397	Ac22:13OH	0.4	0.5
3415	Ac21:14OH	-	0.4
3434	Ac22:13OH	-	0.5
3456	Ac21:14OH	-	0.1
3491	2,(6/8/12)DiMe19Ac:14OH	4.4	5.4
3524	Ac21:14OH	-	0.2
3571	Ac22:14OH	-	0.1
3640	Ac23:13OH	1.5	2.0
3672	Ac24:13OH	0.2	0.2

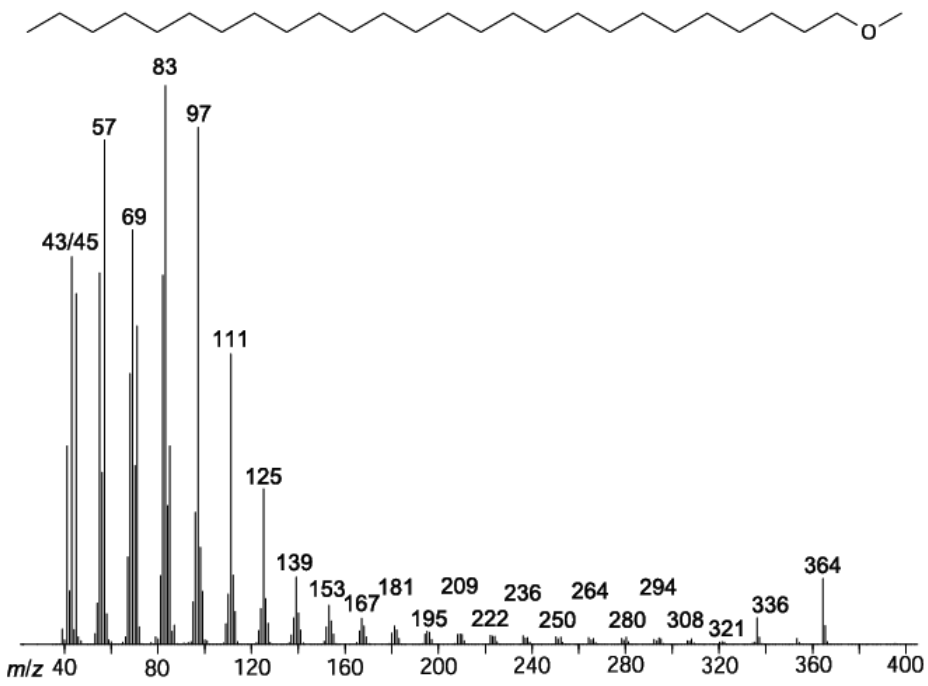

Figure 132: Mass spectrum of 1-methoxyhexacosane.

5.3.5 Chemical composition of web extracts of *Argiope bruennichi* males

Web extracts of virgin and mated males in dichloromethane composed of alkanes, aldehydes, methoxyalkenes, and wax esters. Table 22 summarizes the chemical composition of both extracts and Figure 133 shows the corresponding TICs.

The most abundant compound class represented wax esters consisting of dimethyl branched acids and *n*-alcohols. These derivatives accounted for 42.3% of the TIC in virgin and 54.2% in mated web extracts. They were very similar to the previous discussed wax esters of *A. bruennichi* females and acids with a methyl group at C-2 and an additional methyl group in 6-, 8-, or 12-position were predominant. Their chain length ranged from 17 to 19 carbon atoms and they were mainly esterified with *n*-tridecanol. The isomers with the corresponding dimethyl branched C19-acid were the most abundant representatives in virgin as well as in mated extracts with 14.4% and 18.9%, respectively.

Furthermore, unbranched, monomethyl branched, and dimethyl branched alkanes were present in virgin and mated extracts. The blend of alkanes contributed 36.8% to the TIC of a virgin extract and 30.7% to the corresponding mated one. The most important compound of this lipid class was formed by 2-methyloctacosane (2Me-18:H) with 7.5% in webs of virgin males and 6.0% in the corresponding mated ones.

Furthermore, 1-methoxyalkanes occurred in these extracts and 26:1-OMe with 10.5% and 7.2%, respectively was prevalent as in the methoxyalkane blend of body extracts of *A. bruennichi* males.

Unbranched aldehydes with a chain length between 10 and 22 carbon atoms were also present as a minor group of the lipid blend of virgin and male web extracts. They accounted for 3.8% of the TIC of virgin web extracts and for 2.2% of the TIC of mated web extracts and octadecanal (18:al) exhibited the most abundant compound with 0.9% in virgin and 0.5% in mated web extracts.

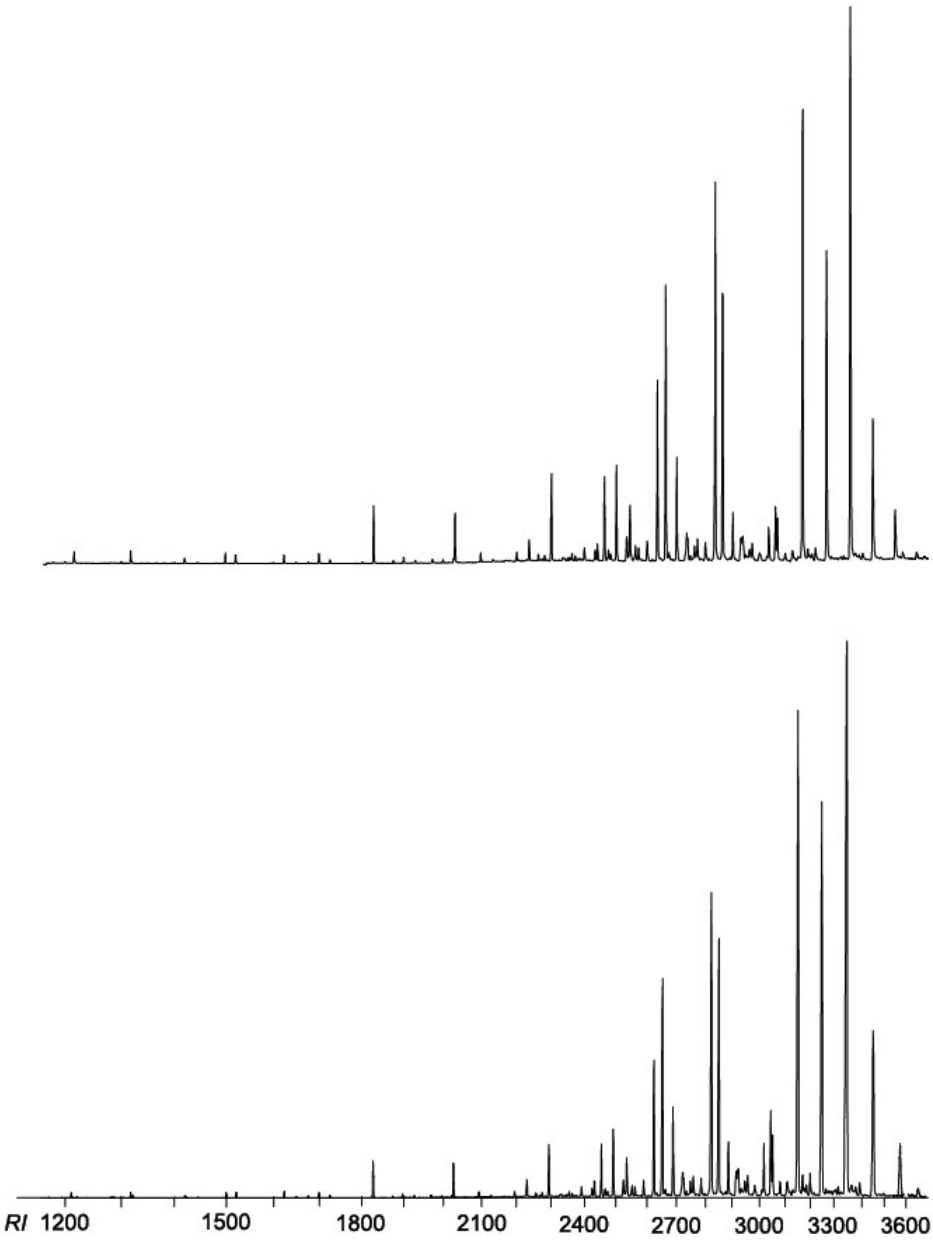

Figure 133: Total ion chromatograms of web extracts of *Argiope bruennichi* males. Top: virgin; bottom: mated.

Table 22: Composition of web extracts of virgin and mated *Argiope bruennichi* males. Intensities of compounds are given in %.

RI	Compound	Virgin	Mated
1200	12:H	Tr	Tr
1216	10:al	0.2	0.1
1300	13:H	Tr	Tr
1317	11:al	0.2	0.1
1400	14:H	Tr	Tr
1419	12:al	0.1	Tr
1500	15:H	0.2	0.1
1521	13:al	0.2	0.1
1600	16:H	Tr	Tr
1623	14:al	0.2	0.1
1700	17:H	0.2	0.1
1725	15:al	Tr	Tr
1800	18:H	Tr	Tr
1827	16:al	1.0	0.6
1900	19:H	0.1	0.1
1930	17:al	0.1	Tr
2000	20:H	0.1	Tr
2016	16:OAc	Tr	-
2032	18:al	0.9	0.5
2100	21:H	0.2	0.1
2134	19:al	0.1	0.1
2162	2Me-22:H	Tr	-
2171	3Me-22:H	Tr	-
2200	22:H	0.2	0.2
2216	18:OAc	Tr	-
2236	20:al	0.4	0.3
2263	2Me-23:H	0.1	0.1
2274	Xene-23:H	0.1	0.1
2281	Xene-23:H	0.1	0.1
2300	23:H	1.7	1.0
2334	11Me-23:H	0.1	0.1
2348	5Me-23:H	0.1	0.1
2362	2Me-23:H	0.2	0.1
2372	3Me-23:H	0.1	0.1
2383	9*Z*-18:amide	0.1	-
2388	9*E*-18:amide	0.1	-
2400	24:H	0.4	0.3
2416	20:OAc	Tr	0.1
2433	22:1-OMe	0.3	0.2
2440	22:al	0.4	0.3

RI	Compound	Virgin	Mated
2462	2Me-24:H	1.9	1.0
2475	Xene-25:H	0.3	0.3
2482	Xene-25:H	0.2	0.2
2500	25:H	2.2	1.4
2540	7Me-25:H	0.1	0.1
2562	2Me-25:H	0.4	0.4
2572	3Me-25:H	0.3	0.2
2600	26:H	0.5	0.4
2618	22:OAc	Tr	0.1
2635	24:1-OMe	3.7	2.6
2663	2Me-26:H	6.2	4.5
2690	Xene-27:H	-	0.1
2700	27:H	2.5	1.8
2730	11/13Me-27:H	1.3	1.1
2735	25:1-OMe	”	”
2738	7Me-27:H	”	”
2748	5Me-27:H	0.1	0.2
2762	2Me-27:H	0.5	0.4
2772	3Me-27:H	0.7	0.6
2800	28:H	0.6	0.5
2834	26:1-OMe	10.5	7.2
2863	2Me-28:H	7.5	6.0
2872	3Me-28:H	-	0.2
2896	Xene-29:H	-	0.1
2900	29:H	1.6	1.3
2929	11/13/15Me-29:H	0.6	0.6
2937	7/9Me-29:H	1.4	1.1
2948	5Me-29:H	0.2	0.2
2952	11,15/11,17DiMe-29:H	0.2	0.5
2962	9,19DiMe-29:H; 2Me-29:H	0.4	0.6
2973	3Me-29:H	0.6	0.5
3000	30:H	0.3	0.5
3004	3,9DiMe-29:H	0.2	-
3028	11/12/13/14/15Me-30:H	0.2	0.2
3035	28:1-OMe	0.9	1.2
3059	2Me-30:H	1.4	1.9
3069	2,(6/8/12)DiMe17Ac:12OH	1.2	1.2
3100	31:H	0.3	0.4
3128	11/13/15Me-31:H	0.4	0.6
3138	7/9Me-31:H	0.1	0.2
3151	11,15/13,17DiMe-31:H	0.2	0.3
3169	2,(6/8/12)DiMe17Ac:13OH	11.2	12.2

RI	Compound	Virgin	Mated
3189	Ac20:13OH	0.2	0.5
3199	Ac20:13OH	0.2	0.3
3207	Ac19:14OH	0.2	0.4
3221	Ac20:13OH	0.4	0.6
3265	2,(6/8/12)DiMe18Ac:13OH	7.7	9.8
3291	Ac20:14OH	0.2	0.3
3312	Ac21:13OH	-	0.2
3321	Ac20:14OH	-	0.1
3327	11/13/15Me-33:H	0.2	0.3
3339	Ac21:13OH	0.1	0.3
3352	13,17/13,19DiMe-33:H	-	0.3
3369	2,(6/8/12)DiMe19Ac:13OH	14.4	18.9
3397	Ac22:13OH	0.4	0.5
3415	Ac21:14OH	-	0.4
3434	Ac22:13OH	-	0.5
3456	Ac21:14OH	-	0.1
3491	2,(6/8/12)DiMe19Ac:14OH	4.4	5.4
3524	Ac21:14OH	-	0.2
3571	Ac22:14OH	-	0.1
3640	Ac23:13OH	1.5	2.0
3672	Ac24:13OH	0.2	0.2

5.3.6 Results of head space experiments with *Argiope bruennichi*

A two-chamber choice test was used to evaluate if virgin *A. bruennichi* males respond more sensitive to webs of virgin females than to those of mated females. These experiments were performed by S. Funke in the lab of PD Dr Uhl from the University of Bonn.

A male is placed between two chambers containing a web of a virgin or a mated female on a filter paper. Based on this setup, several questions were investigated: 1) Which chamber did males choose?, 2) How often did males walk over the filter paper with silk?, 3) Did males vibrate with legs and opisthosoma after silk contact?, 4) How long did a male rest in a female chamber?, and 5) How long did a male take for a decision?

The outstanding presence of trimethyl methylcitrate in body extracts and webs of virgin females and in contrast its absence or decreasing presence in body extracts and webs of subadult as well as mated females led to the hypothesis that this compound is a female pheromone. A positive response of virgin *A. bruennichi* males to virgin webs was observed for the first three questions. Virgin males chose more often the chamber with the web of a virgin female than that of the mated female and this response was significant by the statistical analysis of a Chi-Square test as shown in Table 23.

Table 23: Which chamber did males choose?

Virgin females:	33	73.3%
Mated females:	12	26.7%

significant! (Chi-Square test: $x_1^2 = 9.800$, N = 45, df = 1, p = 0.002)

Furthermore, virgin males walked significantly more often over the web of virgin females than over that of mated females as illustrated in Table 24. This statistical analysis was supported by a Mann-Whitney-U test.

Table 24: How often did males walk over the filter paper with silk?

Virgin females:	6.59 ± 5.13
Mated females:	3.50 ± 2.39

significant! (Mann-Whitney-U test: U = 114.5, Z = -2.172, $N_1 = 12$, $N_2 = 33$, p = 0.03)

Male courtship behaviour as vibrating with the legs and opisthosoma after silk contact was significantly more often displayed on webs of virgin females than on that of mated females though merely 14 of 45 tested individuals showed courtship events during this experiment (Table 25).

Table 25: Did males vibrate with legs and opisthosoma after silk contact?

Virgin females:	12 of 33	36.4%
Mated females:	2 of 12	16.7%

significant! (Chi-Square test: $x_1^2 = 6.422$, N = 45, df = 1, p = 0.011)

No significant responses were obtained for the last two experiments. Males did not spend more time in the chamber with the virgin web than in the other chamber (Table 26) and also the decision for entering into one of the two chambers was independent of the virgin or mated female web (Table 27).

Table 26: How long did a male rest in a female chamber? [min]

Virgin females:	7.51 ± 3.18
Mated females:	7.25 ± 2.81

not significant! (Mann-Whitney-U test: U = 181.000, N_1 = 12, N_2 = 33, Z = -0.449, p = 0.654)

Table 27: How long did a male take for a decision? [min]

Virgin females:	3.59 ± 3.99
Mated females:	5.83 ± 5.49

not significant! (Mann-Whitney-U test: U = 145.500, N_1 = 12, N_2 = 33, Z = -1.363, p = 0.173)

The first experiment demonstrated clearly that a volatile compound must be present on the web of virgin females evoking a preferred response to virgin webs. The result for the second and third experiment showed that the virgin web is more suitable for the induction of courtship display in males and that the web properties are responsible for it because silk contact was essential for the observed behaviour. The insignificant responses for the last two experiments indicated that in addition to the volatile signal, other cues are important for the attractiveness of female webs and for the velocity of the decision.

Based on these experiments, headspace-experiments were performed to investigate if virgin females release compounds which are absent in the corresponding headspace of subadults and mated females. Experiments were carried out by arresting one female in a glass chamber for 24h followed by analysis of its headspace. The glass walls of the chamber were covered with mesh and this mesh supported the locomotion of the female in this chamber and furthermore, it served as a matrix for web construction. Figure 134 shows the TICs of these experiments.

The extracts of subadult and mated females did not contain anything except of artifacts in minor amounts in contrast to the headspace of virgin females. The headspace of virgin females was characterized by trimethyl methylcitrate and thus, this compound is proposed as female pheromone in *A. bruennichi*.

Recent field experiments, performed by S. Funke and PD Dr G. Uhl, have shown that trimethyl methylcitrate is an attraction pheromone for males.

Furthermore, this compound was also identified in the headspace of virgin females of a closely related asian *Argiope* spp. and in web extracts of vigin *A. blanda* and *A. argentata* females which were collected by PD Dr Uhl in Costa Rica.

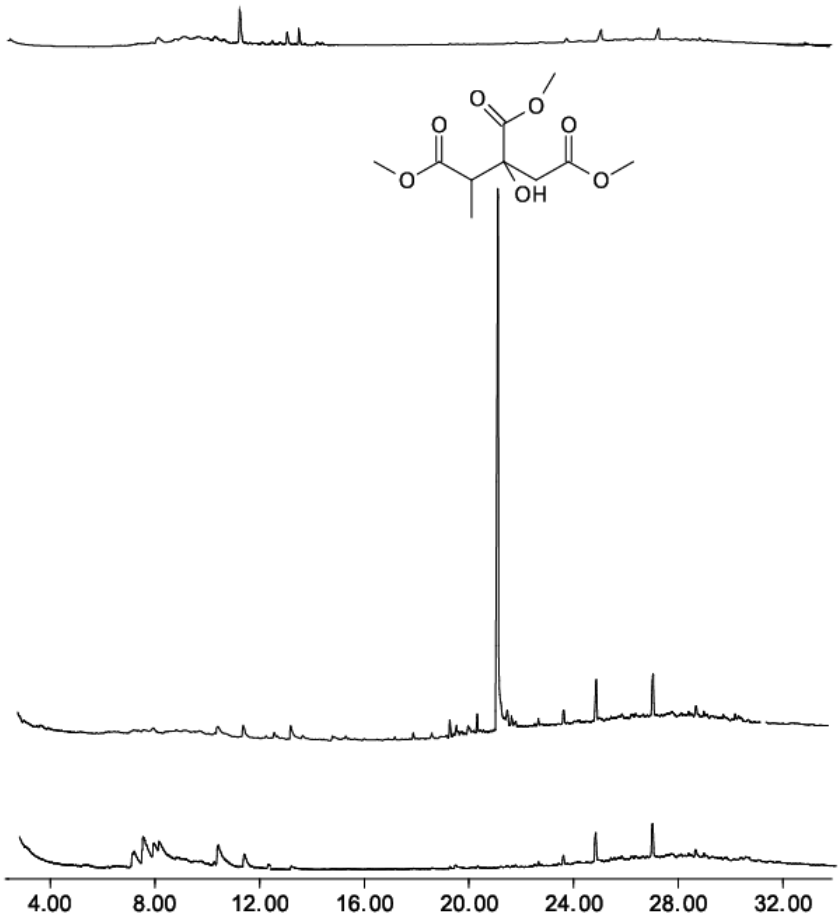

Figure 134: Total ion chromatograms of headspace extracts of *Argiope bruennichi* females. Top: subadult; middle: virgin; bottom mated.

6. Chiral analysis of natural diols

6.1 Methods for the chiral analysis of diols

In general, chiral GC is a suitable method for the determination of the AC of chiral compounds embedded in a complex natural blend. Earlier, deactivated Chirasil-Val columns were reported to be efficient for the chiral analysis of diols [Koppenhoefer *et al.*, 1985; Lin and Koppenhoefer, 1991]. Chirasil-Val columns contain a polysiloxane backbone which is covalently linked to an AA like valine [Frank *et al.*, 1978]. But this phase and also other chiral phases operate merely in a limited temperature range up to 230°C and therefore they are not applicable for the chiral analyses of natural long-chain diols because higher temperatures are necessary for their elutions caused by their high molecular weights.

Alternatively, chiral HPLC-methods exist for the enantiomer separation of 1,2-alkanediols. Different types of vicinal diols were separated as their corresponding 3,5-dinitrophenylcarbamate (3,5-DNPU) derivatives with a separation factor α of at least 1.12 on a chiral stationary phase (CSP) derived by (*R*)-*N*-(2-naphthyl)alanine or (*R*)-*N*-acylated α-aryl-α-aminoalkane [Pirkle *et al.*, 1987]. Later, this method was extended for the separation of enantiomers of long-chain 1,2-alkanediols appearing as diesters with long-chain acids in mammalian skin lipids and bird waxes [Itabashi and Takagi, 1989]. Diols were converted into the corresponding 3,5-DNPU derivatives but instead of a conventional CSP, a chiral slurry-packed capillary column was used for this purpose. Minimum α-values of 1.24 were obtained for their separation by this method.

Indirect methods are also suitable for the determination of the AC and rely on chiral derivatizing agents (CDAs) which transform the enantiomers into diastereoisomers. Diastereoisomers are theoretically separable on achiral phases in HPLC and GC or evoke different chemical shifts in NMR-experiments.

Several applications for the enantiomeric resolution of diols by NMR were described using chiral boronic or phosphinothioic acids. Chiral *t*-butylphenylphosphinothioic acid was applied for the determination of the enantiomeric purity of thiols, amines, diols and amino alcohols [Omelańczuk and Mikolajczyk, 1996]. A resolution $\Delta\delta$ up to 0.01 ppm in ^{1}H-NMR was achieved for some short-chain 1,3-diols by this method. Chiral camphanylboronic acid resolved different diols like short-chain 1,2-diols caused by nonequivalence of signals in ^{13}C-NMR experiments [Tokles and Snyder, 1988]. Recently, enantiomeric diols were converted into diastereomeric acetals by derivatization with axial chiral 2`-methoxy-1,1`-binaphthalene-8-carbaldehyde and these derivatives supported the determination of the AC by NOE-correlations between the protons of CDA and the acetal moiety [Fukui *et al.*, 2003]. The addition of chiral solvents as chiral

1,3-(*p*-methyl-methylbenzyl)-2-methyl-propanediamine to a mixture of enantiomeric diols represents another method by analysis of the $\Delta\delta$-values between the different stereoisomers [Higashibayashi and Kishi, 2004]. But the application of NMR-methods rely on access to enriched or pure samples in a sufficient amount. Therefore, these methods are not suitable for the chiral analysis of diols occurring in complex mixtures in low amounts.

Circular dichroism (CD) exhibits an alternative tool for the determination of the AC of chiral 1,2-diols [Di Bari *et al.*, 2001]. Normally, diols are unsuitable for CD-analysis because they are transparent in the UV-Vis region but coordination to a transition metal with absorption in the UV-Vis region circumvents this restriction. In this case, chiral 1,2-diols coordinate in a ligand-exchange reaction to dimolybdenum tetraacetate followed by recording CD-spectra. The principle of this method is known as Snatzke`s Method and depends on the reliable prediction of the structure of the corresponding complexes [Snatzke *et al.*, 1981; Frelek *et al.*, 1999]. This method provides an empirical rule and the AC of a diol is deduced by the sign of the CD-spectra.

The application of bidentate chiral silylating agents demonstrates another approach for the determination of the AC of chiral diols by GC [Arsene and Schulz, 2002]. Their intention focused on the determination of the AC of 4,6-nonadecanediol (4,6-19:diol) occurring in sunflower pollen. They used (*S*)-[1-(2-bromophenyl)-ethoxy]dichloromethylsilane as CDA in their studies and the AC of the natural compound was assigned to 4*S*,6*R*. It must be mentioned that the number of stereoisomers is doubled by this method because the silicon center becomes chiral. This idea based on the application of enantiomeric pure chloromenthoxydiphenylsilane for the determination of the AC of complex natural alcohols by NMR [Weibel *et al.*, 2000].

6.2 Determination of the absolute configuration of 1,3-docosanediol from the spider *Agelenopsis aperta* and *Heliconius* butterflies

6.2.1 On-column- and bis-TMS-derivatives of acyclic 1,3-alkanediols

During GC-MS-analysis of diol-containing extracts, the conversion of 1,3-alkanediols into on-column-derivatives on BPX-5-phases was ascertained. Their structures were elucidated by the analysis of the corresponding mass spectra and the mass spectrum of the on-column-derivative of 1,3-22:diol is illustrated in Figure 135.

The diols were transformed on column into 2,2-dimethyl-4-alkyl-[1,3,2]-dioxa-silinanes as deduced by the base ion m/z = 131. This ion was formed by cleavage in α-position to the cyclic oxygen atom under release of the alkyl chain. The molecular weight was indirectly derived by the ion $[M - 15]^+$.

But the conversion into the on-column-derivatives was not quantitatively because addition of

MSTFA provided bis-TMS-derivatives and the mass spectrum of the corresponding derivative of 1,3-22:diol is depicted in Figure 135. The mass spectrum was characterized by prominent fragment ions formed by cleavages in α-position to the TMS-moiety. These fragmentations furnished the ions *m*/*z* = 103, 219, and 369 and those proved the 1,3-arrangement of the hydroxy groups in this compound. The ions *m*/*z* = 396 and 471 indicated indirectly the molecular weight of 486 amu and the elimination of trimethylsilanol was responsible for the formation of the first one.

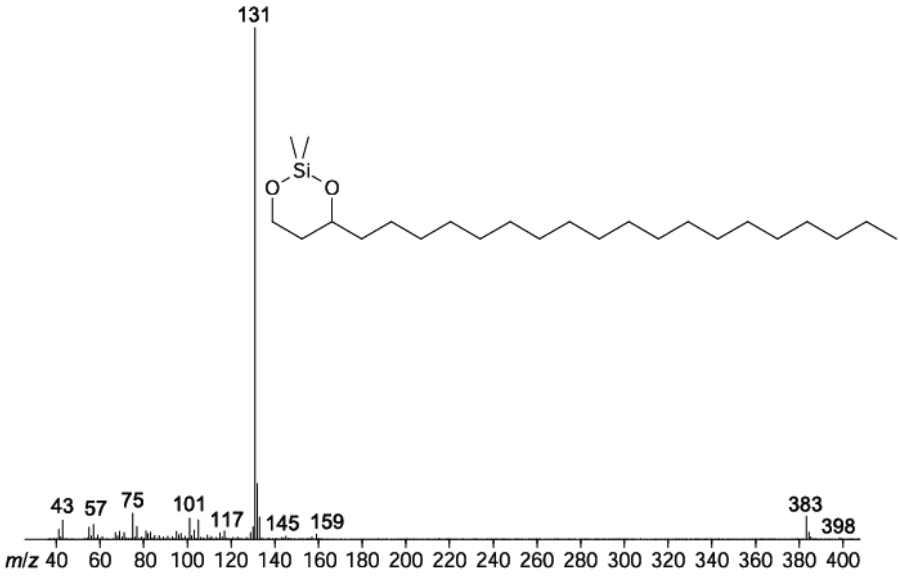

Figure 135: Mass spectrum of the on-column-derivative of 1,3-docosanediol.

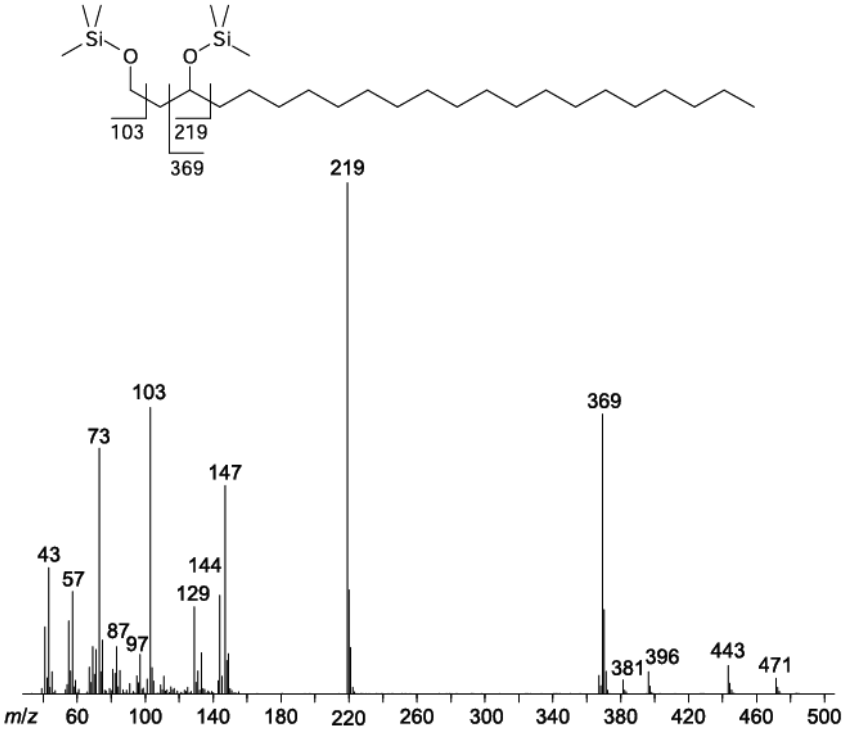

Figure 135: Mass spectrum of the bis-TMS-derivative of 1,3-docosanediol.

6.2.2 Synthesis of enantiomeric pure (*R*)-dichloro-ethyl-methylbenzylsilane

This CDA was synthesized by W. Jacobs in her diploma thesis via an enantioselective hydrosilylation of styrene [Jacobs, 2005].

The enantioselective hydrosilylation of prochiral alkenes is a well-known approach for the synthesis of chiral alcohols in high enantiomeric purities. This method is applicable for the conversion of terminal alkenes, 1,3-dienes, functionalized and non-functionalized styrenes into the corresponding Markovnikov-alcohols [Uozumi *et al.*, 1995; Hayashi *et al.*, 2001; Jensen *et al.*, 2002].

In general, this synthesis is performed in the presence of a silane, di-μ-chlorobis(η^3-allylpalladium) ([PdCl(η^3-C_3H_5)]$_2$) and a chiral ligand which induces a preferred stereochemistry in the corresponding product. Oxidation of the silyl intermediates under Tamao-Fleming-conditions with KF, K_2CO_3 and H_2O_2 in a 1:1-mixture of methanol and tetrahydrofurane yields the corresponding alcohols [Jones and Landais, 1996]. Hayashi`s axial chiral monophosphine ligands (MOPs) are often used as mediators for chirality in these reactions and their common structure is illustrated in Figure 136.

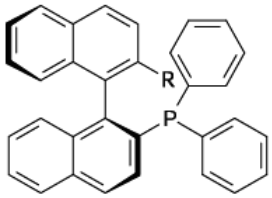

Figure 136: Common structure of Hayashi`s axial chiral monophosphine ligands; R = OMe, H, Ar.

Mechanistic investigations showed that common bidentate phosphine ligands as 2,2`-bis(diphenylphosphino)-1,1`-binaphthalin (BINAP) are unsuitable for the hydrosilylation of alkenes whereas MOPs are appropriate for this purpose. In contrast to the MOP-complexes, the corresponding BINAP-complexes do not evolve any activity caused by a lacking coordination sphere for the alkene.

(*S*)-2-(diphenylphosphino)-2`-methoxy-1,1`binaphthyl (MeO-MOP) was used for the synthesis of 2-alcohols via hydrosilylation of terminal alkenes [Uozumi *et al.*, 1995]. The formation of 2-alcohols is difficult because *anti*-Markovnikov products are favoured [Marciniec, 1992]. Nevertheless, the synthesis of 2-octanol for example was performed with different MOP-ligands in at least 80% yield, 80:20-ratio of regioisomers and 90% enantiomeric excess (ee).

Chiral benzylic alcohols were synthesized by hydrosilylation with Feringa`s ligand illustrated in Figure 137. The obtained ees (up to 99% in 75% - 95% yield) exceeded those with Hayashi`s MOP-ligands [Jensen *et al.*, 2002]. This ligand combines elements of axial and central chirality.

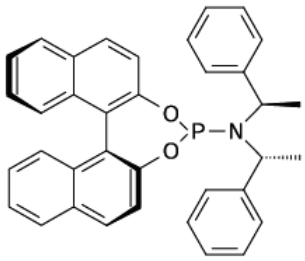

Figure 137: Feringa`s ligand in (S_A,R_C,R_C)-configuration.

Experiments revealed that the enantiomeric excess depends on the AC of Feringa`s ligand because the diasteromer with (R_A,R_C,R_C)-configuration evolves equal activity but products with reduced enantioselectivity are obtained. For example, inversed stereoproducts with (*S*)-configuration and an ee of only 60% were observed.

The synthesis of (*R*)-dichloro-ethyl-methylbenzylsilane **151** was performed by the enantioselective hydrosilylation of styrene in the presence of (a*S*,c*R*,c*R*)-configured Feringa`s catalyst as illustrated in Figure 138.

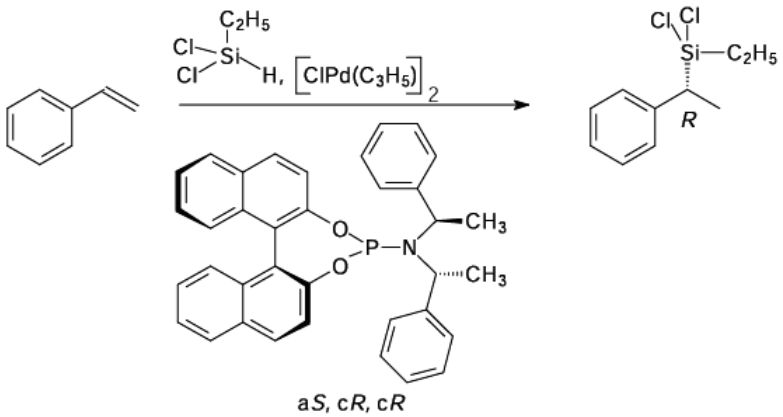

Figure 138: Synthesis of (*R*)-dichloro-ethyl-methylbenzylsilane.

In contrast to the reported excellent yields, **151** was only obtained in 17% yield but the ee was ≥ 99% [Jacobs, 2005].

The (*R*)-configuration of **151** was determined during this thesis by its oxidation to 1-phenyl-ethan-1-ol **152** under Tamao-Fleming conditions followed by chiral GC with *rac*- and (*S*)-**152** as reference compounds. The results of this experiment are shown in Figure 139.

Similar hydrosilylations of stryrene with other substituted silanes in the presence of this ligand furnished also (*R*)-configured products [Jensen *et al.*, 2002]**.**

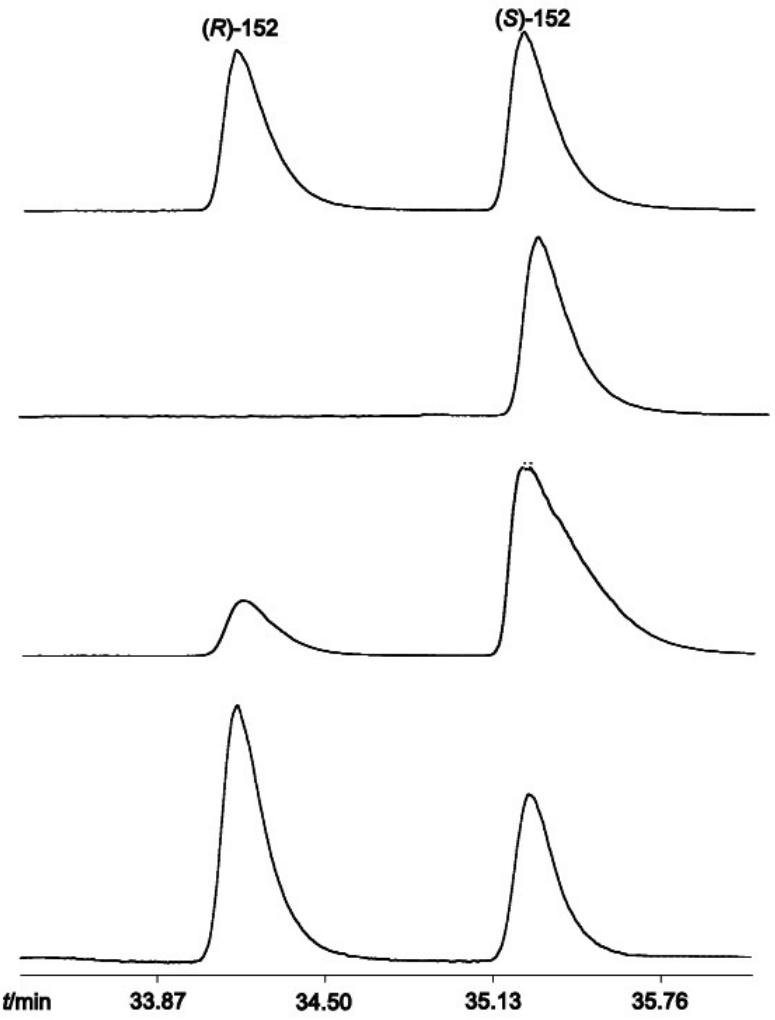

Figure 139: Determination of the absolute configuration of **152**. Top: *rac*-**152**; middle top: (*S*)-**152**; middle bottom: coinjection of *rac*-**152** and (*S*)-**152**; bottom: coinjection of *rac*-**152** and oxidized **151**. 80°C isothermal for 2 min, then 1°C/min, Chiral Phase: hydrodex-6-TBDMS–Phase.

6.2.3 Synthesis of (3*S*)-1,3-docosanediol and its racemate

(*S*)-1,3-Docosanediol (1,3-22:diol) **157** and its racemate **155** were synthesized as reference compounds for the determination of the AC of natural 1,3-22:diol in *A. aperta* and *Heliconius* spp. according to Figure 140. Eicosanal **153** was transformed into the 3-oxo ethyl ester **154** in the presence of ethyl diazoacetate and tin(II) chloride in 78% yield [Holmquist and Roskamp, 1989]. Reduction of **154** with $NaBH_4$ in a refluxing mixture of methanol and dioxane provided the racemic diol **155** in 71% yield [Soai and Oyamada, 1984]. On the other hand, **154** was hydrogenated with enantiomeric pure [*S*(-)-BINAP]-chloro-(*p*-cymene)-ruthenium chloride under formation of the (*S*)-configured *β*-hydroxy methyl ester **156** in 77% yield. Finally, **156** was reduced to **157** with LAH in 97% yield.

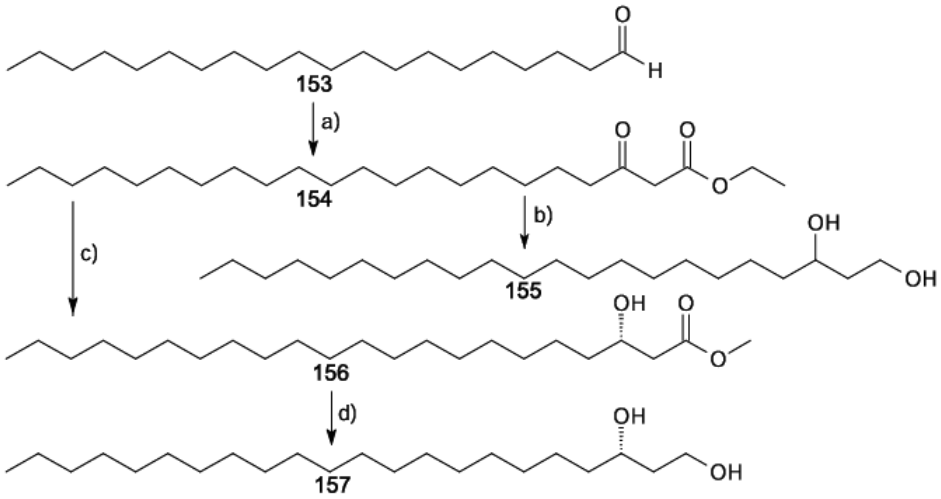

Figure 140: Synthesis of (*S*)-1,3-docosanediol and its racemate. a) diazoacetate, $SnCl_2$, abs. CH_2Cl_2, rt overnight, 78%; b) $NaBH_4$, dioxane, MeOH, 2h reflux, 71%; c) H_2, MeOH, [*S*(-)-BINAP]-chloro-(*p*-cymene)-ruthenium chloride, 15 bar, 80°C, 48h, 72%; d) LAH, Et_2O, 2h reflux, 97%.

6.2.4 Determination of the AC of natural 1,3-docosanediol in *Agelenopsis aperta* and *Heliconius* spp.

Derivatization of 1,3-22:diol with (*R*)-dichloro-ethyl-methylbenzylsilane proceeded rapidly under formation of the [1,3,2]-dioxasilinanes **158**. Figure 141 shows the mass spectrum of these derivatives.

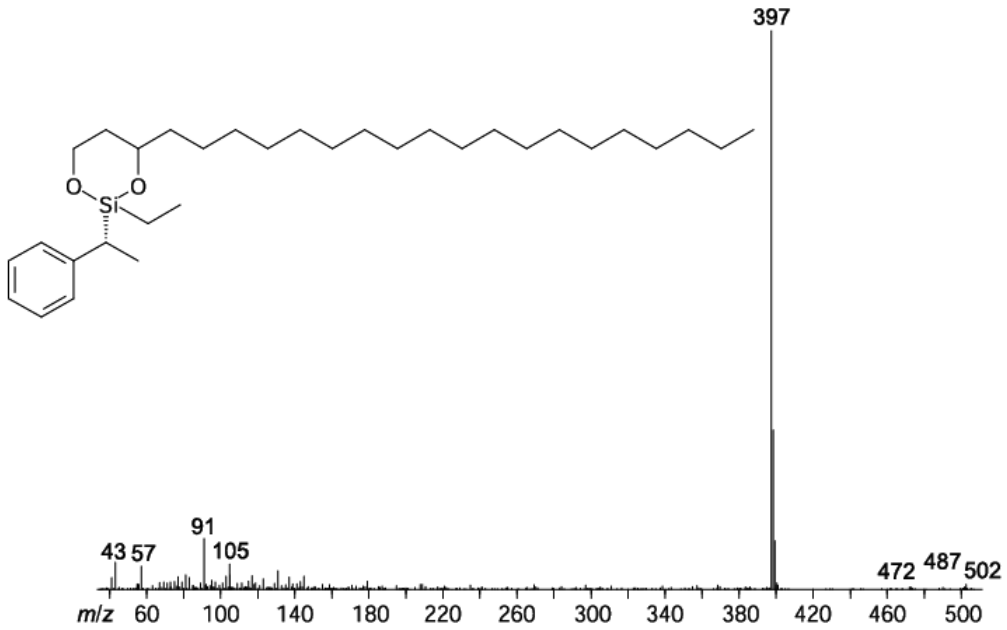

Figure 141: Mass spectrum of the corresponding [1,3,2]-dioxasilinane of 1,3-docosanediol.

The mass spectrum of this derivative was characterized by the base ion *m*/*z* = 397 caused by the cleavage of the methylbenzyl moiety. Other ions occurred only in low intensities. The ions *m*/*z* = 473 and 487 were formed by the loss of an ethyl group and methyl group, respectively from the molecular ion *m*/*z* = 502. The ion *m*/*z* = 397 is suitable as diagnostic ion for the analyses in the single ion mode (SIM).

The derivatization of 1,3-docosanediol furnished four diastereoisomers **158** because the silicon center became chiral during this procedure. The formation of all possible isomers is illustrated in Figure 142.

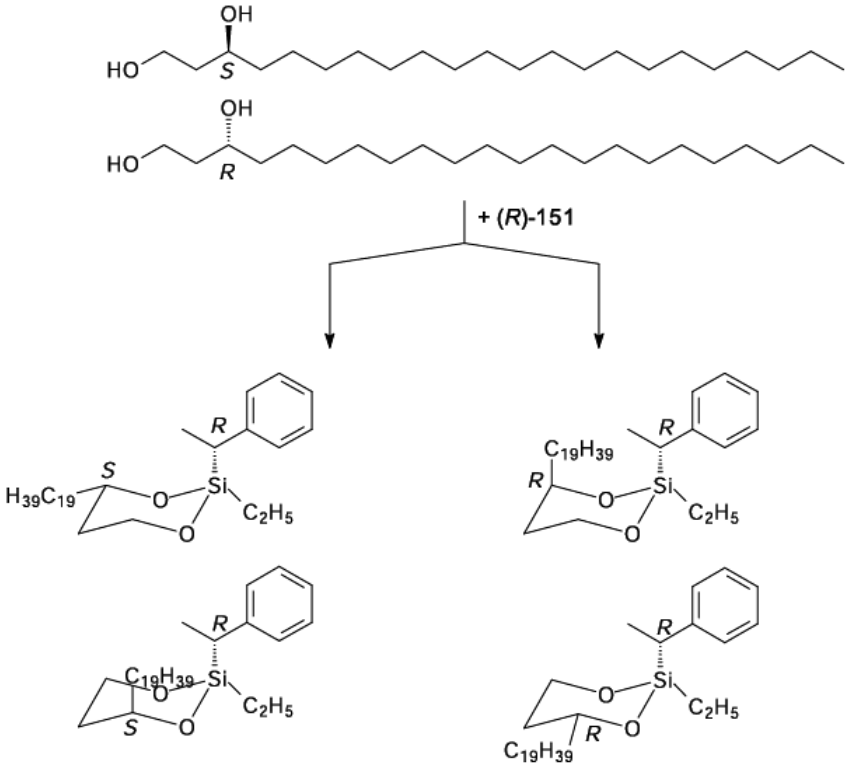

Figure 142: Formation of four diastereoisomers (**158**) during the derivatization of racemic 1,3-docosanediol with (*R*)-**151**.

The results of the chiral analyses of natural 1,3-22:diol in *A. aperta* and *Heliconius* spp. on an achiral BPX-5-phase are summarized in Figure 143.

First, a racemic mixture of 1,3-22:diol was derivatized and a chromatogram with three resolved peaks in an intensity of 2:1:1 were obtained. Theoretically, based on Figure 142, four peaks in a 1:1:1:1-ratio were expected. Thus, two stereoisomers overlapped on this phase under the experimental conditions (2 min 100°C isothermal, 4°C/min up to 320°C). The same result was obtained under different experimental conditions. Analogously, a sample of (3*S*)-1,3-22:diol was derivatized and the corresponding chromatogram demonstrated that both (*S*)-anomers were separated under these conditions. Peak assignment in the chromatogram of the racemic mixture was

performed by coinjection of a 1:1-mixture of *rac*- and (*S*)-**158**. Coinjection showed that a (*S*)- and a (*R*)-anomer coeluted in the first peak. The second peak corresponded to the second (*R*)-anomer and the last peak to the second (*S*)-anomer.

Then, natural extracts of *A. aperta* and *Heliconius* spp. were derivatized and two base-line separated peaks in an approximate ratio of 1:1 were observed in the corresponding chromatograms of both samples. Subsequent coinjection of *rac*-**158** and natural samples in a 1:1-ratio furnished chromatograms containing three peaks in which the first and the last peak were enhanced in intensity compared to the 2:1:1-ratio of the peaks in the chromatogram of the *rac*-**158**. Thus, the natural extracts contained 1,3-22:diol in the (*S*)-configuration and the configuration was equal independent of the arthropod source of this compound.

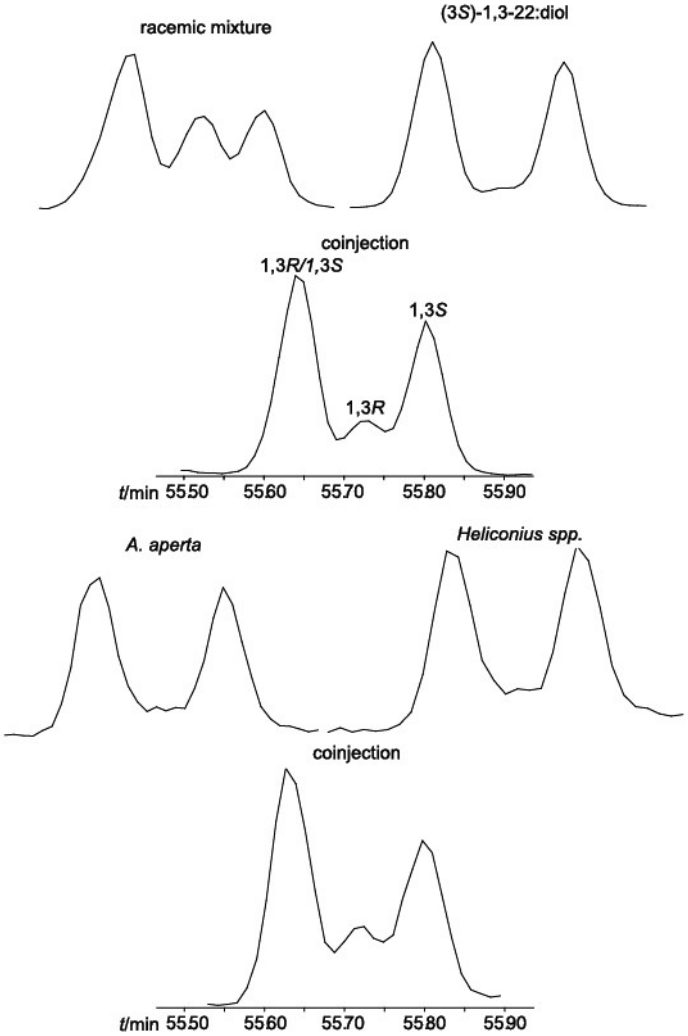

Figure 143: Determination of the AC of natural 1,3-docosanediol by derivatization with (*R*)-**151** in *Agelenopsis aperta* and *Heliconius* spp.. Top left: *rac*-**158**; right; (*S*)-**158**; middle top: coinjection of *rac*- and (*S*)-**158**; middle bottom: left natural sample *Agelenopsis aperta*; right: natural sample *Heliconius* spp.; bottom: coinjection of *rac*-**158** and natural **158**. BPX-5-phase; 2min 100°C isothermal, 4°C/min up to 320°C.

6.3 Analysis of chiral diols with novel C_2-symmetric silylating reagents

6.3.1 Introduction

C_2-symmetric silylating reagents for the analysis of chiral diols were prepared to avoid the formation of anomers during their analyses. For this purpose, C_2-symmetric 1,3-diols **159** were treated with silicon tetrachloride under facile formation of the [1,3,2]-dioxasilinanes **160**. Finally, these intermediates formed the derivatives **161** after addition of the chiral natural diol. Indeed, these derivatives were obtained in microreactions by simultaneous addition of the C_2-symmetric 1,3-diol and the chiral natural diol. This procedure is illustrated in Figure 144.

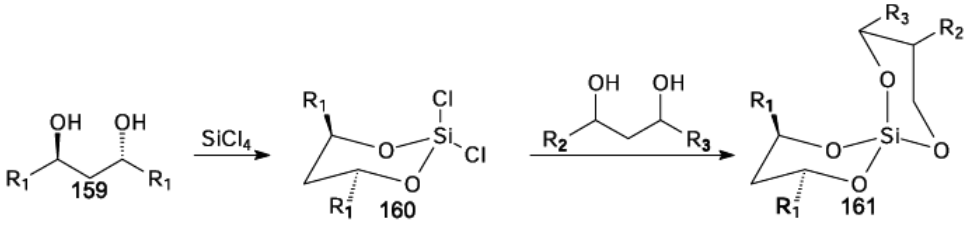

Figure 144: Derivatizing scheme for the analyses of chiral diols with novel C_2-symmetric silylating reagents.

6.3.2 Synthesis of C_2-symmetric diols

Several C_2-symmetric diols differing in the alkyl residues were synthesized according to Figure 145.

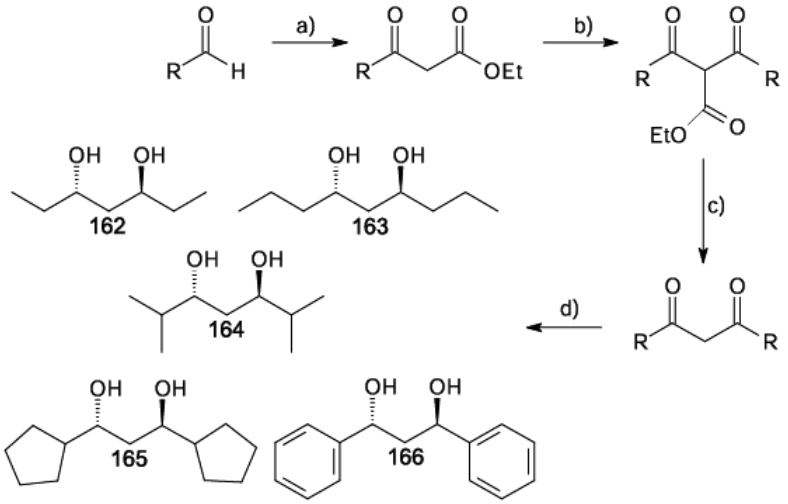

Figure 145: Synthesis of C_2-symmetric diols. a) diazoacetate, $SnCl_2$, abs. CH_2Cl_2; b) RCOCl, pyridine, $MgCl_2$; c) DMSO, NaCl, H_2O, reflux; d) H_2, MeOH, [*S*(-)-BINAP]-chloro-(*p*-cymen)-ruthenium chloride or catalyst preparation according to Taber and Silverberg.

(3*S*,5*S*)-3,5-Heptanediol **162** and (1*R*,3*R*)-1,3-diphenylpropandediol **166** were accessible after enantioselective hydrogenation of the corresponding commercial available diones with a catalyst according to Taber and Silverberg prepared by heating [*S*(-)-BINAP], polymeric 1,5-cyclooctadieneruthenium(II) chloride, triethylamine, and xylene to reflux [Taber and Silverberg, 1991]. This catalyst is stable for several months without loss of activity when stored as saturated THF-solution at -30°C. After a few days, the colour of the solution changes from red to green but nevertheless, its activity is retained. Additionally, this catalyst was also applied for the hydrogenation of the other diones except of the corresponding dione of diol **164**. This dione was hydrogenated with commercial available [*S*(-)-BINAP]-chloro-(*p*-cymene)-ruthenium chloride. Diol **162** was obtained in 72% yield when hydrogenated for 20h with 40bar at 60°C . The AC of **162** was assigned to 3*S*,5*S* by comparison of the optical rotation power with reference data. Its experimental value was characterized by a $[a]_D$ with positive sign analogous to published data for the (3*S*,5*S*)-enantiomer [Rychnovsky *et al.*, 1991]. The corresponding diastereoisomers were only detected in traces in the crude product. The ee of (3*S*,5*S*)-**162** was 100% because the opposite enantiomer was not identified during the chiral analysis of this diol. The ee was determined by derivatization of diastereoisomeric **162** as well as the enantioselective with CDA **160**, formed from (2*R*,4*R*)-pentanediol and silicon tetrachloride. The corresponding GC-MS-analysis of the diastereomeric derivatives showed that those were separated on a BPX-5-phase (5min 50°C, then 10°C/min up to 320°C). Analogously, the sample from the enantioselective synthesis was investigated and only one compound was detected during the analysis.

In contrast, **166** was obtained only in 10% yield in the (1*R*,3*R*)-configuration and this transformation required a reaction time of 24h, 20bar hydrogen atmosphere, and 80°C. The educt was completely consumed but mandelic acid formed the main product in this reaction. A $[a]_D$ with positive sign was obtained as reported for the (1*R*,3*R*)-isomer [Roos and Donovan, 1999]. The ee of this compound has still to be determined because its chiral analysis failed hitherto.

The synthesis of diol **163** started with the acylation of ethyl 3-oxo-hexanaote with butyric acid chloride under formation of ethyl 2-butyryl-3-oxo-hexanoate in 86% yield [Rathke and Cowan, 1985]. Then, ethoxydecarbonylation under Krapcho-conditions furnished 4,6-nonandione in 76% yield [Krapcho, 1982]. Final enantioselective hydrogenation (10bar, 80°C, 24h) provided **163** in the (4*S*,6*S*)-configuration in only 10% yield and its $[a]_D$ was characterized by a positive sign as expected for the (4*S*,6*S*)-isomer [Ohta, 1986].

The corresponding *ß*-keto esters for diols **164** and **165** were obtained in 72% yield by treating the aldehydes with ethyl diazoacetate under Lewis-acid conditions with $Sn(II)Cl_2$ [Holmquist and Roskamp, 1989]. Subsequent acylation furnished the 2-acylated 3-oxo esters in

93% and 63% yield, respectively. Krapcho-deethoxycarbonylation provided the corresponding diones in 64% and 53% yield. Final enantioselective hydrogenation yielded **164** (40bar, 60°C, 20h) and **165** (10bar, 80°C, 20h) in 53% and 48%, respectively. Diol **164** was characterized by a $[a]_D$ with positive sign as described for (3*R*,5*R*)-**164** [Marinetti *et al.*, 1999]. A $[a]_D$ with negative sign was measured for **165** but the AC was not assigned because of lacking reference values for this diol. The determination of the ee for **165** is still in process. The determination of the AC as done for **162** failed because the enantiomers were not separable by this method. In contrast, the ee of **164** was elucidated similar to **162** and the results of these analyses are illustrated in Figure 146.

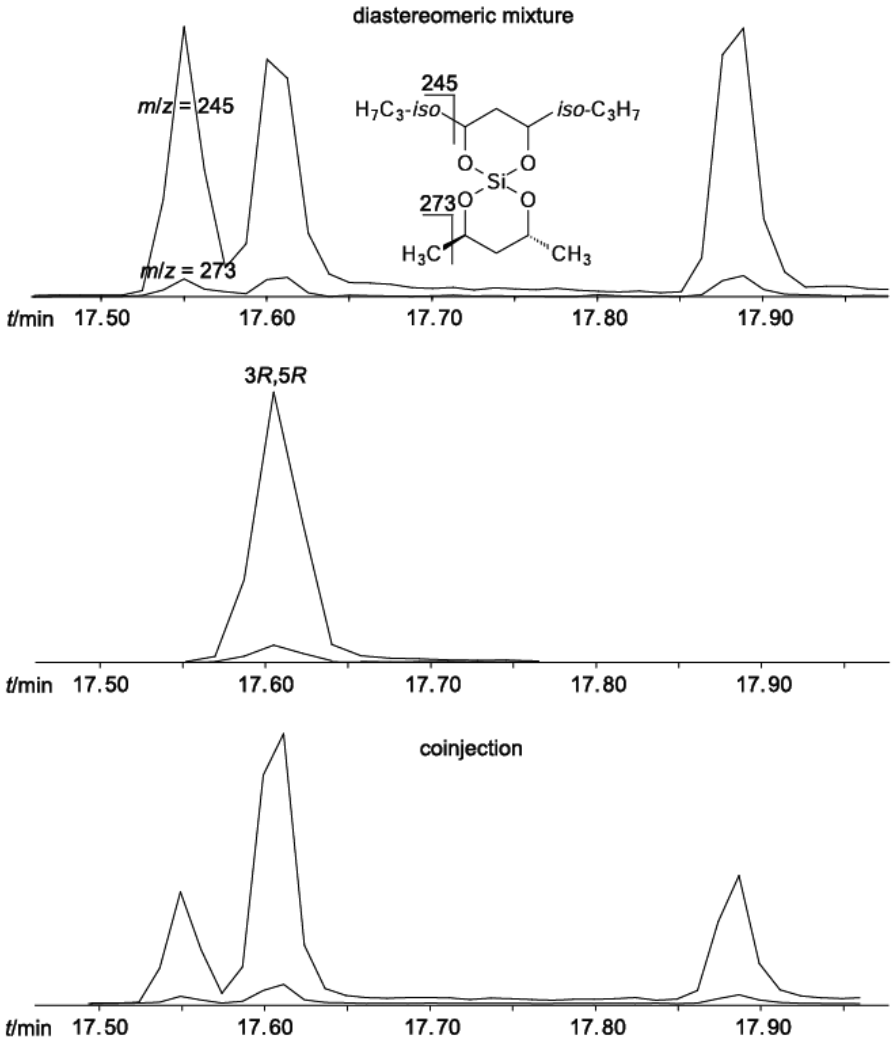

Figure 146: Determination of the enantiomeric excess of (3*R*,5*R*)-2,6-dimethyl-3,5-heptanediol. Top: GC of the corresponding diastereomeric mixture. Middle: GC for the derivative from the enantioselective synthesis. Bottom: coinjection. BPX-5-phase, 5min 50°C isothermal, then 10°C/min up to 320°C.

The figure demonstrates that all isomers were separated by this method on a BPX-5-phase. Only

one derivative occurred in the sample from the enantioselective synthesis and the coinjection indicated that the second peak corresponded to the (3*R*,5*R*)-isomer. The mass spectra of these derivatives were characterzsed by the ions *m*/*z* = 245 and 273. These ions are formed by preferred cleavages in α-position to the rings. Based on this analysis, an ee of 100% was determined for **164**. Table 28 summarizes the applied conditions, the yields, the AC, and the corresponding ORP-values for the syntheses of these diols.

Table 28: Applied conditions for the syntheses of the diols, the corresponding yields, their absolute configurations and ORP-values.

Product	Conditions	Yield	$[a]_D^T$	AC
162	60°C, 40 bar, 20 h	72%	13.0 ± 0.1 (*c* = 3.8, $CHCl_3$), 23°C	(3*S*,5*S*)
163	80°C, 10 bar, 20 h	10%	9.0 ± 0.5 (*c* = 2.0, $CHCl_3$), 23°C	(4*S*,6*S*)
164	60°C, 40 bar, 20 h	52%	54.2 ± 0.1 (*c* = 2.6, MeOH), 23°C	(3*R*,5*R)*
165	80°C, 10 bar, 20 h	48%	-7.2 ± 0.2 (*c* = 2.6, $CHCl_3$), 23°C	-
166	80°C, 20 bar, 24 h	10%	46.1 ± 0.1 (*c* = 2.2, MeOH), 22°C	(1*R*,3*R*)

6.3.3 Chiral analyses of 1,3-diols

These diols were investigated for their ability to separate the stereoisomers of different 1,3-diols. First, the results of the chiral analyses of 2,4-nonanediol are discussed. This compound was used in a *anti*/*syn*-ratio of 2:1 for these analyses. Figure 147 depicts the results of these analyses and Table 29 shows the calculated chromatographic separation factor α ($[(t_{R1} / t_{R2})]$) and the chromatographic resolution R_S ($[(t_{R1} - t_{R2}) / 0.5*(\omega_1 + \omega_2)]$).

Table 29: Calculated resolution R_S and separation factor α for the diastereomeric mixture of 2,4-nonanediol. ω = peak width [min]; *t* = retention time [min].

Ligand	t_{anti1}	t_{anti2}	t_{syn1}	t_{syn2}	ω_{anti1}	ω_{anti2}	ω_{syn1}	ω_{syn2}	α_{anti}	$R_{S,\,anti}$	α_{syn}	$R_{S,\,syn}$
(3*S*,5*S*)-**162**	28.52	29.13	-	-	0.20	0.60	-	-	1.021	1.53	-	-
(4*S*,6*S*)-**163**	31.55	32.12	-	-	0.13	0.51	-	-	1.018	1.78	-	-
(3*R*,5*R*)-**164**	30.11	30.84	-	-	0.23	0.34	-	-	1.024	2.18	-	-
(1*R*,3*R*)-**165**	40.12	40.81	-	-	0.17	0.30	-	-	1.017	2.94	-	-
(1*R*,3*R*)-**166**	45.06	45.65	-	-	0.49	0.25	-	-	1.013	1.59	-	-

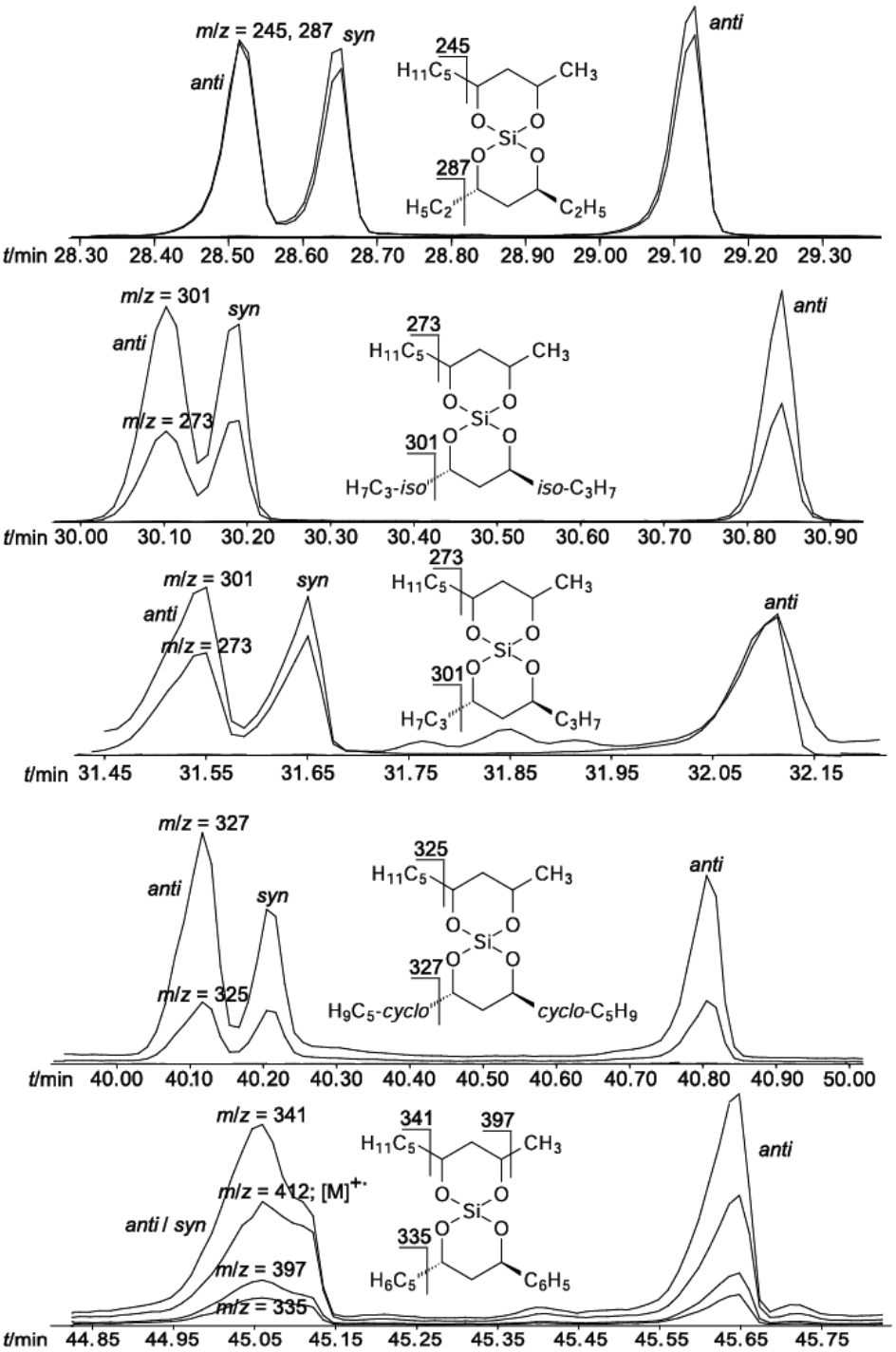

Figure 147: Chiral analyses of 2,4-nonanediol. First: Analysis with (3*S*,5*S*)-3,5-heptanediol; second: analysis with (4*S*,6*S*)-nonanediol; third: analysis with (3*R*,5*R*)-2,6-dimethyl-3,5-heptanediol; fourth: analysis with (1*R*,3*R*)-1,3-dicyclopentylpropanediol; fifth: analysis with (1*R*,3*R*)-1,3-diphenylpropanediol. BPX-5-phase, 2min 50°C then with 5°C/min up to 320°C.

The *anti*-isomers of 2,4-nonanediol were resolved in every case whereas the *syn*-isomers remained unseparated. The highest α-value of 1.021 was obtained in the analysis with the CDA formed from **164** and the lowest with 1.013 for the corresponding one from **166**.

The R_S-value is more important because this value is independent of the retention time *t*. This value takes only into account the distance of peaks and the peak width and distant peaks with a small peak width provide high R_S-values. The analysis with the CDA formed of **165** furnished the highest R_S-value with 2.94 and the lowest one of 1.53 with that of **162**. But in both cases more than a base-line separation was achieved. One *anti*-isomer and a *syn*-isomer coeluted in the derivatization with the CDA formed of **166.**

Furthermore, these analyses were analogously performed with the diastereoisomers of 4,6-nonadecanediol, synthesized by C. Arsene during his PhD thesis. Figure 148 and Table 30 illustrate the obtained results. The diastereomeric mixture was prepared by randomly mixing the enantiomeric pure *anti*-isomers and a racemate of the *syn*-isomers.

Table 30: Calculated chromatographic resolution R_S and separation factor α for the chiral analyses of the diastereomeric mixture of 4,6-nonadecanediol. ω = peak width [min]; *t* = retention time [min].

Ligand	t_{anti1}	t_{anti2}	t_{syn1}	t_{syn2}	ω_{anti1}	ω_{anti2}	ω_{syn1}	ω_{syn2}	α_{anti}	$R_{S,\,anti}$	α_{syn}	$R_{S,\,syn}$
(3*S*,5*S*)-**190**	44.18	44.62	-	-	0.23	0.30	-	-	1.010	1.66	-	-
(4*S*,6*S*)-**191**	45.87	46.34	-	-	0.13	0.26	-	-	1.010	2.41	-	-
(3*R*,5*R*)-**192**	45.07	45.64	-	-	0.29	0.27	-	-	1.013	2.04	-	-
(1*R*,3*R*)-**193**	52.37	52.94	52.45	52.49	0.14	0.17	0.05	0.16	1.011	3.68	1.001	0.38
(1*R*,3*R*)-**194**	56.02	56.70	56.28	56.36	0.27	0.33	0.15	0.19	1.012	2.27	1.001	0.47

The CDAs formed from the diols **164**, **165**, and **166** were capable to separate all stereoisomers of 4,6-nonadecanediol, indicated by two local maxima for the *syn*-isomers in the corresponding GCs whereas the other ones were just able to resolve the *anti*-isomers. These two local maxima were not recognized as two separated peaks in the GC for the CDA formed from diol **164** and thus data for the resolution of the *syn*-isomers are lacking in Table 30. The *anti*-isomers were base-line separated with every CDA. The α-values for the separation of the *anti*-isomers differed just slightly and they ranged from 1.010 to 1.013 and the CDA formed from **164** furnished the highest value. The corresponding R_S-values ranged from 1.66 to 3.68 and the highest value was obtained for the CDA formed from **165**. These values underscore the base-line separation of the *anti*-isomers because they are $\geq$ 1.5 [Baugh, 1993]. The CDA formed from **166** was the most

suitable reagent for the chiral analyses because besides base-line separation of the *anti*-isomers, the *syn*-isomers were separated with the highest R_S-value of 0.47. Thus, this reagent and also those formed from **164** and **165** facilitate the chiral analysis of this diastereomeric mixture compared to the previous applied (*S*)-[1-(2-bromophenyl)-ethoxy]dichloromethylsilane.

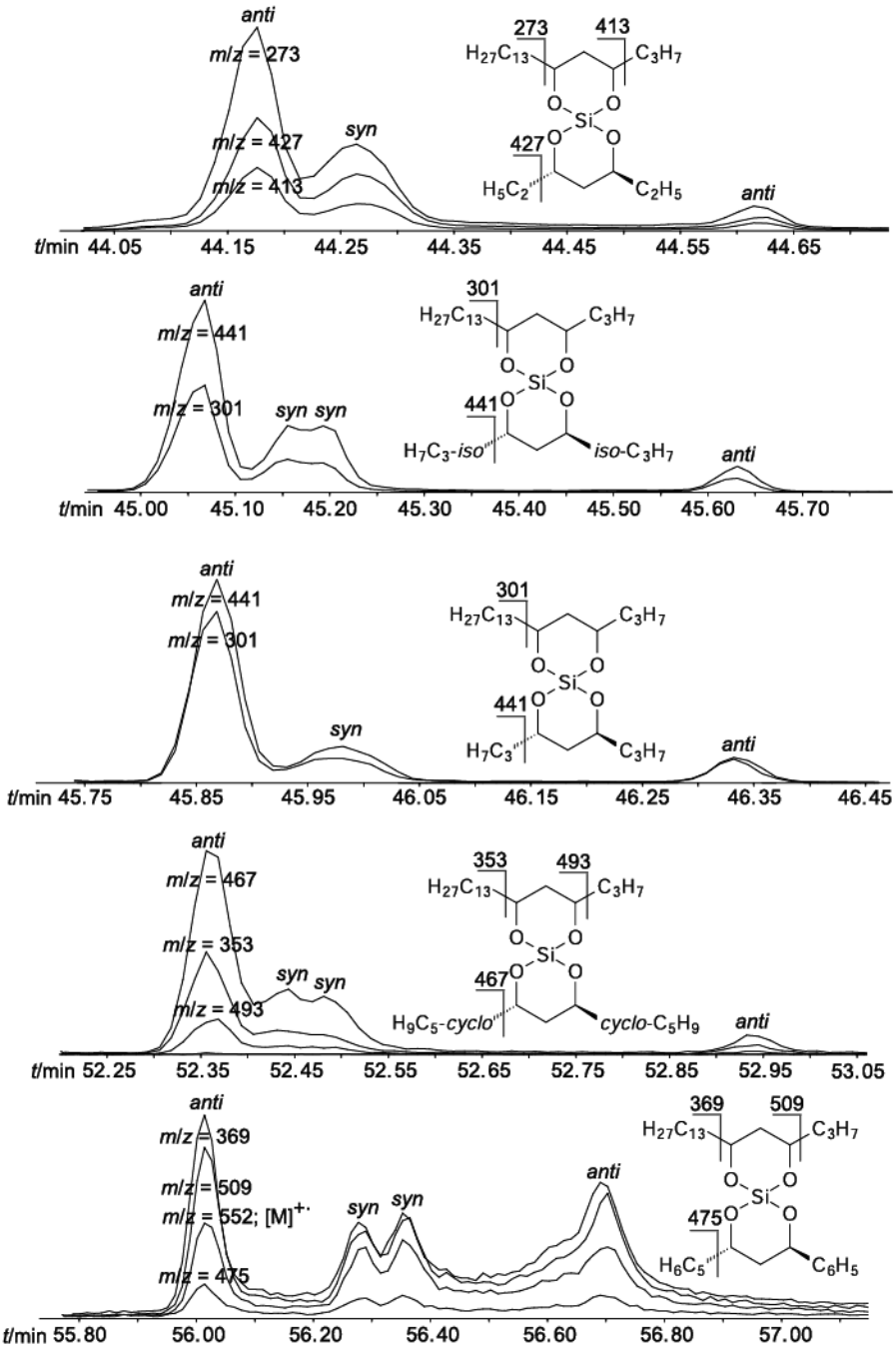

Figure 148: Chiral analyses of 4,6-nonadecanediol. First: Analysis with (3*S*,5*S*)-3,5-heptanediol; second: analysis with (4*S*,6*S*)-4,6-nonanediol; third: analysis with (3*R*,5*R*)-2,6-dimethyl-3,5-heptanediol; fourth: analysis with (1*R*,3*R*)-1,3-dicyclopentylpropanediol; fifth: analysis with (1*R*,3*R*)-1,3-diphenylpropanediol. BPX-5-phase, 2min 50°C isothermal, then with 5°C/min up to 320°C.

In addition, the separation properties of these CDAs for the resolution of the diastereomers of 7,9-heptacosanediol, synthesized by S. Yildizhan, was investigated. But as observed in the experiments for 2,4-nonanediol, merely the *anti*-isomers were separable. Figure 149 shows some of the results of these experiments and those were not influenced by the temperature conditions during the experiments because different starting temperatures and steep as well as flat temperature ramps in the non-isothermal phase provided similar results.

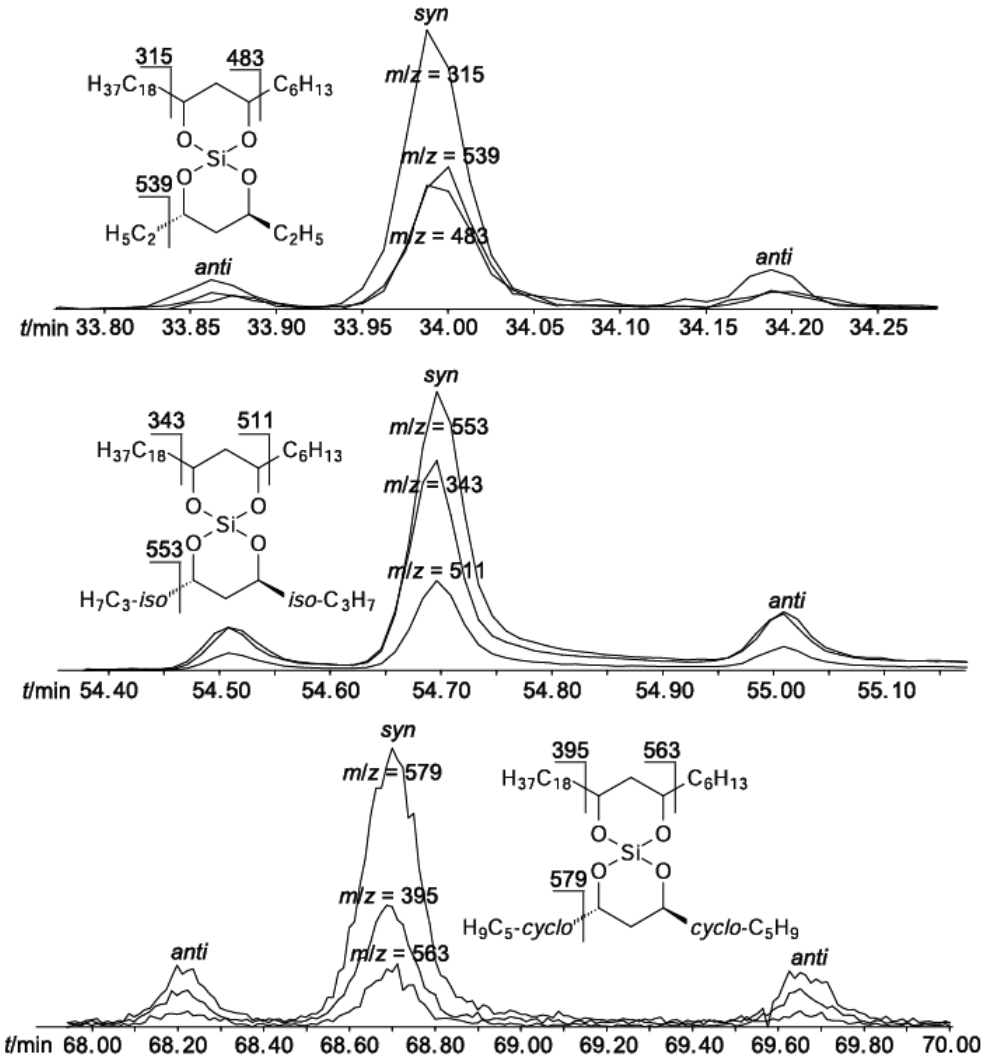

Figure 149: Chiral analyses of 7,9-heptacosanediol. First: Analysis with (3*S*,5*S*)-3,5-heptanediol (5min 50°C, then 10°C/min up to 320°C); second: analysis with (3*R*,5*R*)-2,6-dimethyl-3,5-heptanediol (1min 50°C, then 5°C/min up to 320°C); third: analysis with (1*R*,3*R*)-1,3-dicyclopentylpropanediol; 5min 100°C, then 4°C/min up to 320°C ; BPX-5-phase.

6.3.4 Chiral analyses of other diols

(2*R*,3*R*)-hexanediol and (2*S*,3*R*)-hexanediol were identified as male sex pheromones of the longhorn beetles *Hylotrupes bajulus* and *Pyrrhidium sanguineum* [Schröder *et al.*, 1994] Earlier, the potential of these CDAs for the resolution of the enantiomers of 2,3-hexanediol was investigated [Goller, 2003]. During this experiment all stereoisomers were resolved on a BPX-5-phase. Analogously, this method was applied for the chiral analyses of 7,8-heptacosanediol, but the corresponding derivatives were not formed under different experimental conditions. In contrast, derivatization with (*R*)-**151** was possible but its separation properties were not sufficient for the chiral analysis.

Furthermore, the analysis of chiral 1,4-diols was investigated and therefore 2,5-decanediol in a diastereomeric ratio (d.r.) of 1.3:1 was used. The results of the GC-MS-analyses are shown in Figure 150 and Table 31 describes the corresponding α- and R_S-value for the experiment with the CDA formed from **162**.

Table 31: Calculated chromatographic resolution R_S and separation factor α for the chiral analysis of the diastereomeric mixture of 2,5-decanediol. ω = peak width [min]; t = retention time [min].

Ligand	t_{EP1}	t_{EP1}	t_{EP2}	t_{EP2}	ω_{EP1}	ω_{EP1}	ω_{EP2}	ω_{EP2}	α_{EP1}	$R_{S,EP1}$	α_{EP2}	$R_{S,\ EP2}$
(3*S*,5*S*)-**162**	29.52	30.20	30.34	30.44	0.26	0.24	0.13	0.16	1.023	2.72	1.003	0.69

The experiments with the CDAs formed from **164** and **166** showed two resolved peaks and the remainig isomers coeluted but a shoulder in this peak was observed. Interestingly, the ratio of the used diastereomeric mixture of 2,5-decanediol was not reflected during the chiral analysis and the ratios deviated from that of initially determined d.r of 1.3:1. Perhaps, the formation of some derivatives is favoured whereas others are disfavoured. The distribution of stereoisomers in the experiment with the CDA formed from **162** corresponded to the initial d.r. and furthermore, all stereisomers were resolved. An α- of 1.023 and a R_S-value of 2.72 were obtained for the first enantiomeric pair and thus base-line separation was achieved. The second enantiomeric pair was nearly base-line separated and an α- of 1.003 and a R_S -value of 0.69 were calculated. These results and those obtained for the diastereoisomers of 2,3-hexanediol demonstrate the potential of these CDA for the stereoisomer separation of short-chain 1,2- and 1,4-diols. Chiral short-chain diols can be analysed on chiral phases but their analysis after derivatization with CDAs of type **160** exhibits a serious alternative because analysis times are moderate and a widespread applied BPX-5-phase can

be used.

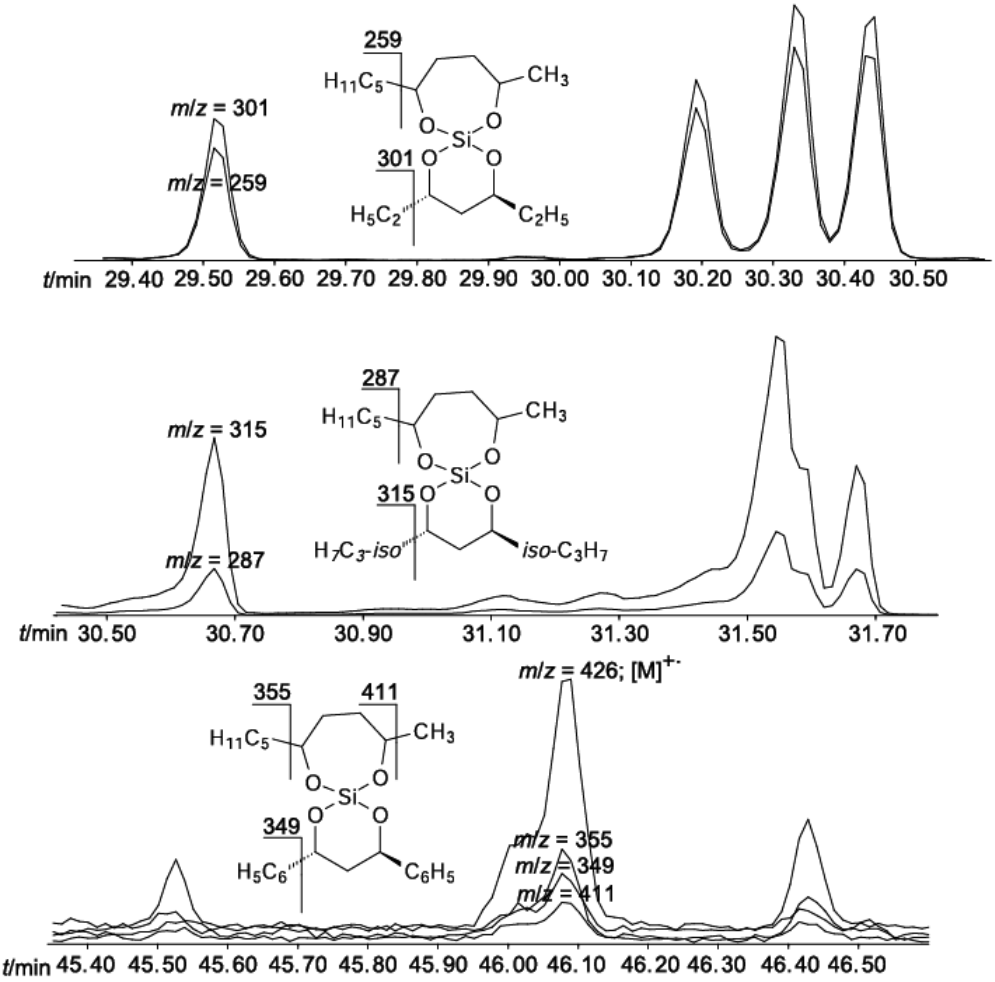

Figure 150: Chiral analyses of 2,5-decaneediol. First: analysis with (3*S*,5*S*)-3,5-heptanediol; second: analysis with (3*R*,5*R*)-2,6-dimethyl-3,5-heptanediol; third: analysis with (1*R*,3*R*)-1,3-diphenylpropanediol; BPX-5-phase; 2min 50°C, then 5°C/min up to 320°C.

7. Summary

Chapter 2 dealt with a comparative analysis of the cuticle lipid blend of male and female *Ampulex compressa*. Chemical analysis revealed that the lipid pattern differed between the sexes. The composition of the male cuticle as well as that of the female cuticle were characterized by unbranched and methyl branched saturated hydrocarbons. Furthermore, unsaturated hydrocarbons occurred. The blends contained alkenes with a chain length between 25 and 29 carbon atoms. The double bonds were present in position 5, 6, 7, 8, and 9. In addition, alkadienes with a all-*cis*-configured 6,9-arrangement of the double bonds were identified in the female cuticle extract. (6*Z*,9*Z*)-6,9-heptacosadiene (6*Z*,9*Z*-27:H) represented the most abundant compound of this extract and its structure was elucidated by a combination of microderivatization and synthesis. This compound was also present in the female Dufour gland, but only in minor amounts. The composition of the female cuticle and the Dufour gland were similar. Bioassays with a synthetic 6*Z*,9*Z*-27:H are planned to investigate if this compound is involved in the semiochemistry of this species.

All investigated extracts of the Dufour gland contained 2-methyl-3-pentanone as the only volatile compound. Other compounds than hydrocarbons were identified in the lipid blend of the male cuticle. Alkyl benzoates with a chain length of 18, 20, or 22 carbon atoms were present and these derivatives were hitherto unknown in arthropods. Furthermore, (*Z*)-3-nonenal (3*Z*-9:al) was identified as trace compound in this lipid blend. In general, 3*Z*-9:al is unstable. But it seemed to be very stable in the male cuticle extract because it was still present in the extract after time. Here, 3*Z*-9:al is described for the first time as natural compound in arthropods. Similarly to the results of Swedenborg (1992), the formation of 3*Z*-9:al may be explained by oxidative degradation of 6*Z*,9*Z*-27:H. The formation of 3*Z*-9:al by oxidation of synthetic 6*Z*,9*Z*-27:H under UV-irradiation was shown. Furthermore, 17-methylhentriacontan-10-one occurred as major representative of a blend of methyl branched ketones in the male lipids. The position of the methyl branch was elucidated after transformation to the corresponding monodeuterated alkane. Therefore, the ketone was initially converted to a tosyl hydrazone, followed by reduction with a deuterium source, and subsequent analysis of its mass spectrum. This method opens a new possibility to determine the position of methyl branches in long-chain aliphatic ketones occurring in complex mixtures of natural compounds.

In addition, the volatiles of the male and female digger wasp *Liris niger* were analysed. 3,6-Dimethyl-2-isobutylpyrazine was identified in the head space of unmated females, also present in the head space of mated females, but only in minute amounts, and this compound evoked a strong response of the male antenna in electrophysiological experiments. The pyrazine was absent in

males. Now, a bioassay must show if this compound is able to elicit a behavioural response in males. A gland in the head region is responsible for the formation of the pyrazine but the exact site is still unknown. Furthermore, the male antenna responded to contents of the Dufour gland. Some of the tested hydrocarbons were able to evoke a response of the male antenna but also hydrophilic compounds of this gland are recognized. Future work is directed to a more detailed chemical analysis of the Dufour gland.

The identification of novel spider lipids was reported in chapter 3. A sexual dimorphism in the composition of the cuticular lipid blend was demonstrated in *Argyrodes elevatus*. The lipid blend of the male cuticle contained one major compound, undecyl 2-methyltridecanoate. The structure of this compound was identified by a combination of derivatization methods. Analogously, four major wax esters, 2-methylundecyl 2,8-dimethylundecanoate, 2,8-dimethylundecyl 2,8-dimethylundecanoate, heptadecyl 4-methylheptanoate, and 14-methylheptadecyl 4-methylheptanoate, were identified in the lipid blend of the female cuticle. In contrast to insects, the role of the constituents of the cuticular lipid blend for the semiochemistry of spiders is not well understood and therefore, based on the strong sexual dimorphism in the lipid patterns, this organism is a useful model to study their function.
New methoxyalkanes with the methoxy group at C-2 were identified in the dwarf spider *Diplocephalus permixtus*. The cuticular lipids differed from those of closely related species, which contained only 1-methoxyalkanes. The 2-methoxyalkanes were featured by one or three methyl branches. The fact that the methyl branches occured in even-numbered positions indicated a new biosynthetic pathway. Besides these 2-methoxyalkanes, the isomeric 1-methoxyalkanes were present pointing to a biosynthetic pathway responsible for the formation of all methoxyalkanes.

Chapter 4 dealt with the investigation of the biosynthesis of (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene (3*Z*,6*Z*,9*Z*-18:H), an even-numbered type II lepidopteren pheromone. It is a compound of the pheromone blend of the winter moth *Erannis bajaria*. In contrast to the odd-numbered related structures, the biosynthesis of the even-numbered hydrocarbons was unkown. Recently, it was proposed that these compounds are formed by reductive modifications of the corresponding even-numbered acids via aldehydes and alcohols as intermediates [Millar, 2000]. This was disproved because (9*Z*,12*Z*,15*Z*)-1,1,2,2-tetradeuterooctadeca-9,12,15-trien-1-ol, applied as precursor to *E. bajaria*, furnished no deuterated 3*Z*,6*Z*,9*Z*-18:H. On the other hand, deuterated 3*Z*,6*Z*,9*Z*-18:H was obtained after feeding of (10*Z*,13*Z*,16*Z*)-2,2,3,3-tetradeuterononadeca-10,13,16-trienoic acid (D_4-10*Z*,13*Z*,16*Z*-19:COOH) and D_4-11*Z*,14*Z*,17*Z*-20:COOH, demonstrating

that ubiquitous α-linolenoic acid is first elongated to 11*Z*,14*Z*,17*Z*-20:COOH followed by α-oxidation under formation of 10*Z*,13*Z*,16*Z*-19:COOH. Finally, decarbonylation or decarboxylation provides 3*Z*,6*Z*,9*Z*-18:H.

Furthermore, a novel analysis of pheromone gland extracts of calling *E. bajaria* revealed an altered pheromone blend. Usually, females of this species produce only type II pheromones and a 1:1-mixture of 3*Z*,6*Z*,9*Z*-18:H and 3*Z*,6*Z*,9*Z*-19:H attracted males in field tests [Plaß, 1996]. But recent analyses showed a shift to compounds, resembling more type I pheromones. The hexane extract of the pheromone gland was now dominated by (*Z*)-12-heptadecen-2-one, (*Z*)-11-heptadecenal and (*Z*)-1,12-heptadecadiene. Currently, field tests are performed by G. Szöcs to clarify if these compounds have a function in the semiochemistry of this species.

In chapter 5, head space experiments for the identification of the catalepsis pheromone of the male desert spider *Agelenopsis aperta* were performed. Derivatives of γ-butyrolactones and δ-valerolactones were identified in the head space of males which were absent in that of females. These compounds were also present in the male/female and the male/female web experiments. Besides these lactones, (*E*)-2-configured aldehydes were identified as male-specific compounds. These derivatives occurred also in the male/female and the male/female web experiments. A similar distribution profile was observed for unbranched 2-ketones. Representatives of theses compound classes are tested in the laboratory of Dr S. Riechert by A. Payne for their capability to induce catalepsis in unmated females. Furthermore, other interesting compounds were identified in the head space of males and females. One of these compounds was farnesylacetaldyde, an irregular terpene aldehyde with 17 carbon atoms. In addition, two pyrroles, *N*-3-methylbutylpyrrole and 3-methyl-*N*-3-methylbutylpyrrole, occurred in the volatile blends, both hitherto unknown as natural products in arthropods.

The chemical analyses of the wasp spider *Argiope bruennichi* demonstrated two general patterns which support the assignment of the developmental stage of the males and females. First, dichloromethane extracts of the body of subadult females contain a 1:1-mixture of (*Z*)-9- and (*E*)-9-octadecenamide. The ratio of this mixture changes in favour of the (*E*)-isomer in the corresponding extracts of adult females. Analogously, this 1:1-mixture was observed in the body extracts of subadult males and similarly, the ratio of this mixture changed in adult males. Furthermore, the analyses of dichloromethane body and web extracts showed that extracts of virgin females contained trimethyl methylcitrate in a diastereomeric ratio of 25:1 (in average). The stereochemistry of these compounds are still unknown. Both were absent in the corresponding extracts of subadult females and were also absent or present only in traces in those of mated

females. According to the sex pheromone of the tropical ctenid spider *Cupiennius salei*, (*S*)-dimethyl citrate, it was investigated if the diastereoisomers of trimethyl methylcitrate are sex pheromones in *A. bruennichi*. A two-chamber choice test with webs of mated and unmated females demonstrated that adult virgin males preferred significantly the web of unmated females [Funke and Uhl; personal communication]. In addition, male contact with webs of unmated females was significantly longer than the contact with webs of mated females. Based on these results, a volatile compound was proposed to be responsible for the long-range attraction of the males to webs of unmated females. Subsequent head space experiments revealed that trimethyl methylcitrate was the only compound produced in higher amounts in the volatile blend of virgin females compared to the head space of subadult and mated females. Recently, a bioassay with a diastereomeric mixture of trimethyl methylcitrate led to male attraction in the field indicating the importance of trimethyl methylcitrate in the semiochemistry of this species [Funke and Uhl; personal communication] .

New methods for the stereochemical analysis of diols were investigated in chapter 6. The (*S*)-configuration of natural 1,3-docosanediol in the desert spider *A. aperta* and *Heliconius* butterflies was determined by the use of (*R*)-dichloro-ethyl-methylbenzylsilane. The stereochemical analyses of internal 1,3-diols with this reagent furnished no positive results.
Furthermore, chiral silylating reagents based on the utilization of C_2-symmetric diols as ligands were developed for the stereochemical analyses of internal diols. These reagents are able to separate all stereoisomers of short-chain 1,2- and 1,4-diols in GC-experiments on a BPX-5-phase, as shown earlier for the diastereoisomers of 2,3-hexanediol and here for 2,5-decanediol. These diols can also be analysed by chiral GC, but the method development is more difficult than the chiral analysis on a widespread applied BPX-5-phase. The suitability of these reagents for the stereochemical analyses of short-chain- and long-chain 1,3-diols is not predictable. The separation of the *anti*-isomers was possible in every case, while it failed for the separation of the *syn*-isomers in the case of 2,4-nonanediol and 7,9-heptacosanediol. On the other hand, the *syn*-isomers of 4,6-nonadecanediol were separated by silylating reagents bearing isopropyl, cyclopentyl, or phenyl groups. Nevertheless, it is worth to apply this method if the stereochemical analysis of a 1,3-diol is needed because a broad spectrum of C_2-symmetric diols is accessible by the synthesis presented here. Furthermore, this derivatization procedure is easy to handle and in addition, other serious alternatives for the analysis of these diols in low amounts in complex mixtures, except of the method applied by Arsene and Schulz (2002), are lacking.

8. Experimental section

8.1 General methods

Syntheses

Chemicals were purchased from Fluka Chemie GmbH (Buchs, Switzerland), Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and Acros Organics (Geel, Belgium). The chemicals were used without further purification. Solvents were purified and dried according to standard methods [Perrin, Armarego, and Perrin, 1980]. Glass-ware was oven-dried before and syntheses were carried out under an inert atmosphere of nitrogen if necessary.

Thin-layer chromatography (TLC)

TLC were performed on 0.2 mm pre-coated plastic sheets Polygram Sil G/UV_{254} (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Plates were developed by soaking them into a bath of 0.5 g molybdato phosphorc acid in 100 mL of ethanol followed by warming them with a heat gun. The same procedure was performed with a bath of potassium permanganate (5 g $KMnO_4$, 20 g K_2CO_3, 5 mL 1N NaOH, and 300 mL H_2O) or a vanillin bath (8.6 g vanillin, 2.5 mL H_2SO_4, and 200 mL ethanol). Alternatively, UV-active compounds were detected with an UV-lamp.

Column chromatography

Crude products were purified by silica gel chromatography (Merck silica gel60) with overpressure. Dichloromethane, diethyl ether, ethyl acetate, hexane, and pentane were used as solvents for this purpose.

Hydrogenations

Hydrogenations were carried out in an autoclave HR-100 (Berghof Company, Eningen, Germany). The temperature during hydrogenations was controlled with the theromstat BAR 945 (Berghof Company, Eningen, Germany). Hydrogenations up to 40 bar were performed with this device.

Polarimetry

The optical rotation power (ORP) of chiral compounds was measured with a Dr Kernchen Propol Digital Automatic Polarimeter (Dr Kernchen Company, Seelze, Germany). Measurements were carried out in a 1 mL cell at a wavelength of 589 nm. Specific ORPs were determined by formation

the average value of 10 measurements and calculation of the corresponding standard deviations.

GC-MS

GC-MS was carried out on a HP 6890 Series GC system connected to a HP 5973 Mass Selective Detector (Hewlett-Packard Company, Wilmington, USA) fitted with a BPX-5 fused-silica capillary column (l = 25 m, d_i = 0.22 mm, 0.25 µm film, SGE Inc., Melbourne, Australia). Instrument parameters were adjusted as follows: inlet pressure 77.1 kPa; He 23.3 ml/min; injector 250 °C; injection volume 1 µl; transfer line 300°C; electron energy 70eV. Synthetic samples were analysed in split mode (ratio 20:1) whereas the injector was operated in splitless mode (60 s valve time) for the investigation of natural extracts. *RI*s of natural compounds under non-isothermal conditions were determined from a homologous series of *n*-alkanes. Compounds were identified by comparison of their mass spectra with those of mass spectral libraries (Wiley 7 Library; Essential Oils Library, Massfinder) or synthetic references.

Chiral GC

Chiral GC was performed on a GC 8000 Top (CE Instruments, Wigan, England) fitted with a hydrodex-6-TBDMS–Phase with a maximum temperature of 220 °C (l = 15 m, d_i = 0.25 mm, and 0.15 µm film). Hydrogen was applied as carrier gas and compounds were detected with a flame ionization detector. Injector temperature 250°C; detector temperature 300°C; injection volume 1 µl; inlet pressure 80.0 kPa; carrier gas flow 1 mL/min; gas velocity 29 cm/sec.

NMR-Spectroscopy

^{1}H-NMR and ^{13}C-NMR spectra with trimethylsilane as internal standard were recorded on a DPX-200, AVII-300, or DRX-400 (Bruker Daltonik GmbH, Bremen). Compounds were dissolved in deuterated solvents. If necessary, 2D-NMR-experiments were performed for unambiguous structure elucidation.

Microreactions

Microreactions were carried out in 1 mL or 4 mL vials and were heated with the block heater QBD1 (Grant Instruments, Cambridge, England). Microreactions at rt were shaked with the lab shaker Promax 1020 (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany). The corresponding work-up procedures are mentioned in the next section.

8.2 Preparation of natural extracts

Extract preparation by invasive methods

Natural extracts were prepared by soaking a suitable number of individuals of a species in solvents like pentane, CH_2Cl_2, or methanol. SupraSolv solvents (Merck KGaA, Darmstadt, Germany) were used for this purpose.

SPME

A suitable SPME-fiber was conditioned in the injection port of a GC at 320°C for 45 min meanwhile animals were put into boxes and stored for a few minutes in a refrigerator at 4°C. Then, cuticular material of the inactive animals was wiped off with the SPME-fiber followed by GC-MS-analysis.

Open loop stripping analyses (OLSA) for the headspace-analyses of arthropods

General setup of the experiments:

A cleaned and oven-dried glass chamber in which the animals were kept during these experiments was connected to two charcoal filters. These filter were washed several times with CH_2Cl_2 before use. One of these filters was attached in front of the glass chamber whereas the other was placed behind the chamber. Furthermore, the latter one was directly linked to a pump which continously sucked air through the glass chamber over an appropriate time interval. The first filter cleaned the air which passed the glass chamber to avoid accumulation of contaminants on the second filter. After finishing the experiment, the second filter was extracted several times with CH_2Cl_2 followed by concentration of the extract to a volume of 1 to 5 µL. Blank runs were performed before and between the experiments to rule out the transfer of volatiles between the experiments.

8.3 Derivatizations

Derivatizations with DMDS

DMDS-adducts were obtained by stirring equal amounts of freshly distilled DMDS and natural extract, dissolved in pentane, with 1/10 of the volume of a 5% I_2-solution in diethyl ether at 60°C for overnight. After that, excess I_2 was removed with an aqueous solution of Na_2SO_3. The organic phase was separated in a 1 mL syringe and the aqueous phase was extracted twice with 100 µL of pentane. The combined organic phases were dried over $MgSO_4$ followed by removing the solvent. The dried extract was taken up in an appropriate amount of solvent followd by GC-MS-analysis.

Derivatization with nicotinic acid

Nicotinic esters of alcohols were formed by treating the natural extract, dissolved in CH_2Cl_2, with 1 mg of nicotinic acid, 1 mg of EDC and a granule of DMAP, followed by shaking at rt for 2h. The extract was dried by a gentle stream of nitrogen and then taken up in a solvent followed by GC-MS-analysis.

Derivatizations with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA)

About 50 µL of MSTFA were added to the dry extract or a solution of the natural extract in pentane followed by heating for 1h at 60°C. Then, the extract was dried by removing excess solvent and MSTFA under nitrogen. Finally, the dried extract was dissolved in a solvent and analysed by GC-MS.

Derivatization with sodium pyridylmethanolate

A 3% (w/w) stock solution of sodium pyridylmethanolate was prepared by dissolving sodium completely in pyridylmethanol at 60°C in a block heater. The natural extract in pentane (20 µL) was treated with two drops of the resulting paste followed by warming at 60°C for 3h in a block heater. Then, 200 µL of methanol and pentane were added, the extract was shaken, and the pentane phase was separated in a 1 mL syringe. The methanol phase was extracted twice with 200 µL of pentane and the pentane phases were combined. After that, NaCl was added to the pentane extract to remove last traces of excess pyridyl methanolate. Finally, the extract was dried and taken up in a solvent for the GC-MS-analysis.

Derivatizations with trimethylsulfonium hydroxide (TMSH)

Transesterifications were carried out by the addition of 50 µL of TMSH to 10-20 µL of the natural extract followed by heating the sample at 60°C for 2h with a block heater. Then, the extract was dried with nitrogen and the extract was dissolved in a solvent suitable for the GC-MS-analysis.

Derivatization with (*R*)-dichloro-ethyl-methylbenzylsilane

A stock solution of the reagent was prepared by dissolving 10 mg in 1 mL toluene. About 10 µL of the reagent were added to 20 µL of the natural extract and then, the mixture was heated at 60 °C for 2h. Excess reagent was removed with nitrogen followed by dissolving the residue in a suitable solvent. Finally, this extract was analysed by GC-MS.

Derivatization of chiral diols with *C*$_2$-symmetric diols and silicon tetrachloride

This transformation was carried out by mixing solutions of the chiral diol, the C_2-symmetric diol, DMAP, pyridine, and silicon tetrachloride in toluene and heating the mixture for overnight at 100°C. Then, the extract was dried and taken up in diethyl ether. The resulting suspension was filtered with silica gel in a pipette column followed by concentration to an appropriate volume for the GC-MS-analysis.

Deoxygenation of ketones

p-Tosylhydrazone (1 mg) was added to an extract of the ketones in ethanol followed by stirring at rt for overnight. The solvent was removed, the residue taken up in diethyl ether, filtered with silica gel in a pipette column, and concentrated to a final volume of 50 µL. This extract was dried with nitrogen and then redissolved in $CHCl_3$ followed by addition of catecholborane [Kabalka and Summers, 1981]. This mixture was shaken for 1h at rt before 20 µL of [D_1]-methanol and 40 µL of [D_6]-dimethylsulfoxide were added followed by heating at 80°C for 1h. Then, 200 µL of D_2O were added and the organic phase was extracted three times with CH_2Cl_2 in a 1 mL syringe. The organic phases were combined, dried over $MgSO_4$, and concentrated to an appropriate volume for GC-MS-analysis.

Formation of alkyl cyanides

The natural extract (50 µL) and an equal amount of trimethylsilyl iodide (TMSI) were heated at 70°C for 2h followed by removing excess reagent with nitrogen. The residue was taken up in 100 µL CH_2Cl_2, 1 mg of tetraethylammonium cyanide was added, and the mixture was shaken at rt for overnight. Then, 100 µL H_2O were added. The organic phase was extracted three times with CH_2Cl_2 and dried over $MgSO_4$. Finally, the extract was analysed by GC-MS.

Oxidation with ruthenium tetroxide

A vial was filled with a mixture of 50 µL CCl_4, 50 µL acetonitrile, 75 µL H_2O, and 10 mg sodium metaperiodate, followed by the addition of the natural extract (10 µL) and 1 mg ruthenium trichloride hydrate [Carlsen *et al.*, 1981]. The mixture was shaken at rt before 250 µL CH_2Cl_2 were added. The organic phase was separated with a 1 mL syringe and the aqueous phase was extracted twice. The organic phases were combined and the solvent removed. The residue was taken up in diethyl ether, filtered with silica gel in a pipette column and concentrated to an appropriate volume for GC-MS-analysis.

Photochemical degradation of (6*Z*,9*Z*)-6,9-heptacosadiene (9)

In a NMR-tube, a solution of 0.006 g (0.16 mmol) **9** in 800 µL hexane was prepared and the sample was treated with a slight stream of air under UV-irradiation. Samples were taken every hour and analysed by GC-MS.

8.4 Syntheses

8.4.1 General procedures

A:Esterification of acids with EDC [Patel *et al.*, 1998]

One equiv. acid, 1 equiv. alcohol, and 0.1 equiv. DMAP were dissolved in an appropriate amount of anhydr. CH_2Cl_2, cooled to 0°C, and 1.1 equiv. EDC were added in portions. This mixture was stirred overnight, water and CH_2Cl_2 were added, and the organic phase was separated. The aqueous layer was washed twice with CH_2Cl_2, the organic phases were combined, and dried with $MgSO_4$. After concentration, the crude product was purified by column chromatography on silica gel with a mixture of pentane and diethyl ether in a ratio of 5:1.

B: Formation of ω-bromo-alcohols [Jayasuriya *et al.*, 1990]

Five mL 48% aqueous HBr-solution per 1 g diol and 1.5 mL water were mixed and heated under reflux. After a few minutes, a brown organic layer occurred and the progress of the reaction was monitored by TLC. The organic layer was separated, dried over $MgSO_4$, diluted with CH_2Cl_2, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 1/1). The ω-bromo-alcohols were characterized by an average R_F-value of 0.2 in this solvent system.

C: Formation of tetrahydropyranyl ethers [Parham and Anderson, 1948]

One equiv. of the corresponding alcohol and 0.1 equiv. *p*-TsOH were dissolved in an appropriate amount of anhydr. diethyl ether followed by the addition of 1 equiv. 3,4-dihydro-2*H*-pyran. This mixture was stirred at rt overnight. A saturated solution of $NaHCO_3$ was added and the organic phase was separated. The aqueous phase was washed twice with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 5/1). The products were characterized by an average R_F-value of 0.4 in this solvent system.

D: Deprotection of tetrahydropyranyl ethers

One equiv. of a THP-ether was dissolved in methanol and 0.1 equiv. *p*-TsOH were added. This mixture was stirred overnight followed by addition of a saturated solution of $NaHCO_3$ and diethyl ether. The organic phase was separated and the aqueous layer was washed twice with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. Crude products were purified by column chromatography on silica gel.

E: Formation of actetates

Acetyl chloride (1.4 equiv.) and 1.4 equiv. pyridine were dissolved in an appropriate amount of anhydr. CH_2Cl_2 and the mixture was cooled to 0°C. The corresponding alcohol (1 equiv.) was added, the mixture was allowed to warm up to rt, and stirring was continued for 2h. An appropriate amount of H_2O and 2N aqueous HCl-solution were added followed by separation of the organic layer. The aqueous layer was washed twice with CH_2Cl_2, the organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. Crude products were purified by column chromatography on silica gel (pentane/diethyl ether 5/1) and were charaterized by a R_F-value of 0.6.

F: Reduction of acids and their corresponding esters with lithium aluminium hydride (LAH)

A suspension of 1.75 equiv. LAH in an appropriate amount of anhydr. diethyl ether was prepared and cooled to 0°C, followed by addition of a solution of 1 equiv. of the corresponding acid or their ester derivative in anhydr. diethyl ether. The suspension was allowed to warm up to rt and the progress of the reaction was controlled by TLC. After the consumption of the educt was completed, a 2N aqueous HCl-solution was added till two clear phases were formed and the organic phase was separated. The aqueous layer was washed twice with diethyl ether and the organic phases were combined. Then, the organic phase was dried with $MgSO_4$ and the solvents were removed under reduced pressure. Crude products were purified by column chromatography on silica gel.

G: Formation of alkyl cyanides [Taber and Kong, 1997]

Anhydr. acetonitrile (2.5 equiv.) were dissolved in an appropriate amount of anhydr. THF, cooled to -78°C, followed by the addition of 2.5 equiv. *n*-BuLi-solution in hexane. This mixture was stirred for 2h at this temperature and then a solution of 1 equiv. alkyl chloride/bromide in an appropriate amount of THF was added in one portion. Stirring was continued at this temperature for 1h, the mixture was allowed to warm up to rt, and was stirred overnight. The mixture was hydrolized with

H_2O and diethyl ether was added. The organic phase was separated and the aqueous phase was washed twice with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 10/1).

H: Conversion of alkyl cyanides to corresponding aldehydes by reduction with diisobutylaluminium hydride

A solution of 1 equiv. Alkyl cyanide in an appropriate amount of anhydr. toluene was prepared, cooled to 0°C, followed by the addition of 1.35 equiv. of a 1M DIBALH-solution in toluene. The progress of the reaction was controlled by TLC. After the educt was completely consumed, the mixture was cooled to 0°C, and H_2O was added. Furthermore, the reaction was quenched with 2.5% aqueous H_2SO_4-solution and stirring was continued for 30min at this temperature. The mixture was diluted with diethyl ether and the organic phase was separated. The aqueous phase was washed twice with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel.

I: Synthesis of secondary alcohols

One equiv. aldehyde was dissolved in an appropriate amount of anhydr. THF and the resulting mixture was cooled to 0°C. A solution of 3 equiv. methylmagnesium chloride in THF was added dropwise and the mixture was allowed to warm up to rt. Stirring was continued overnight, the mixture was hydrolized with a 2M aqueous HCl-solution, diethyl ether was added, and the organic phase was separated. The aqueous phase was washed twice with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel.

J: Formation of *N*-alkylpyrroles [Brockmann and Tour, 1995]

A solution of 1 equiv. pyrrole, 1 equiv. tetrabutylammonium bromide, and 1.1 equiv. alkylbromide in an appropriate amount of CH_2Cl_2 was prepared, cooled to 0°C, followed by the addition of 1.33 mL/mmol 20% aqueous sodium hydroxide solution. The mixture was heated to reflux for 24h and was quenched with H_2O. The organic layer was separated and the aqueous phase was washed twice with CH_2Cl_2. The combined organic phases were washed with 2N aqueous HCl-solution, H_2O, brine, and were then dried with $MgSO_4$. The crude product was purified by column chromatography on silica gel.

K: Formation of acid amides

A solution of 1 equiv. acid was dissolved in an appropriate amount of anhydr. CH_2Cl_2 . Two drops of anhydr. DMF were added, the mixture was cooled to 0°C, followed by the addition of 2 equiv. oxalyl chloride. Cooling was continued until the production of gas stopped and then, the mixture was stirred for 1h at rt. The solvent and excess oxalyl chloride were removed by destillation, the residue was taken up in an appropriate amount of anhydr. CH_2Cl_2, followed by the addition of excess aqueous solution of ammonia. The mixture was stirred for 30 min at rt, conc. HCl was added, and the solution was diluted with CH_2Cl_2. The organic phase was separated and the aqueous phase was washed twice with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silics gel.

L: Formation of 3-oxo-alkyl esters

Ethyl diazoacetate (1.1 equiv.) was added dropwise to a suspension of anhydr. tin(II) chloride (0.1 equiv.) in anhydr. CH_2Cl_2 (2 mL/mmol aldehyde). A solution of the corresponding aldehyde in anhydr. CH_2Cl_2 (1 mL/mmol aldehyde) was added over a period of 30 min to this mixture. The suspension was stirred for 3h at rt and the mixture was quenched with brine. The organic phase was separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel.

M: Formation of 3-oxo-2-(1-oxo-alkyl)alkyl esters [Rathke and Cowan, 1985]

A suspensionof 1 equiv. anhydr. $MgCl_2$ in 1 mL/mmol anhydr. CH_2Cl_2 was prepared and 1 equiv. of the corresponding 3-oxo-alkyl ester was added. This heterogenous mixture was cooled to 0°C, 2 equiv. anhydr. pyridine were added, and the mixture was stirred for 15 min at this temperature. Then, 1 equiv. of the corresponding acid chloride was added at 0°C and the mixture was stirred for 15 min. The mixture was allowed to warm up to rt and stirring was continued for 2h. The mixture was recooled to 0°C and quenched with 6M aqueous HCl-solution. The mixture was diluted with an appropriate amount of diethyl ether and the organic phase was separated. The aqueous phase was washed twice with diethyl ether. The combined organic phases were dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel.

N: Formation of 1,3-diones [Krapcho, 1982]

A mixture of 1 equiv. 3-oxo-2-(1-oxo-alkyl)alkyl esters, 1 equiv. NaCl, 0.06 mL/mmol H_2O and 1 mL/mmol DMSO was heated to reflux. The progress of the reaction was monitored by TLC and the reaction was stopped after the educt was completely consumed. The mixture was diluted with H_2O and diethyl ether and the organic phase was separated. The aqueous phase was washed twice with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel.

8.4.2 Syntheses of reference compounds

Synthesis of (9*Z*,12*Z*)-9,12-octadecadien-1-ol (7)

This compound was synthesized according to the general procedure **F**.

Used amounts: 0.950 g (25.0 mmol) LAH, 4 g (13.6 mmol) methyl (9*Z*,12*Z*)-9,12-linoleate, and 100 mL anhydr. diethyl ether, yield: 86% (3.135 g, 11.7 mmol).

R_F = 0.13 (pentane/diethyl ether 5/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.9 Hz, 3H, CH_3), 1.26 – 1.40 (m, 18H, 9*CH_2), 1.53 – 1.58 (m, 2H, CH_2), 1.72 (br. s, 1H, OH), 2.05 (q, ^3J = 6.9 Hz, 4H, 2*CH_2), 3.63 (t, ^3J = 6.7 Hz, 2H, CH_2), 5.30 – 5.41 (m, 4H, 4*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.6 (CH_2), 25.7 (2*CH_2), 27.2 (2*CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 31.5 (CH_2), 32.8 (CH_2), 63.0 (CH_2), 128.0 (2*CH), 130.1 (CH), 130.2 (CH).

EI-MS (70eV): *m*/*z* (%) = 266 (5) [M^+], 248 (1) [M^+-H_2O], 135 (11), 121 (18), 109 (22), 95 (52), 81 (76), 67 (100), 55 (70), 41 (87).

Synthesis of (9*Z*,12*Z*)-1-bromooctadeca-9,12-diene (8)

Triphenylphospane (0.587 g, 2.24 mmol) was dissolved in 10 mL of anhydr. CH_2Cl_2, the solution was cooled to 0°C, and 0.11 mL (0.343 g, 2.14 mmol) bromine in 3 mL of anhydr. CH_2Cl_2 were added [Sonnet, 1976]. A white precipitate was immediately formed and the addition of bromine was continued until the white suspension became slight yellow. Excess bromine was removed by addition of triphenylphospane until the suspension became white. Then, a solution of 0.423 g (1.59 mmol) **7** in 10 ml of anhydr. CH_2Cl_2 was added dropwise to the mixture and the cooling bath was removed after the addition was finished. Stirring was continued overnight followed by addition of diethyl ether and H_2O. The organic phase was separated and the aqueous layer was washed

further two times with diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to furnish **8** in 55% yield (0.286 g, 0.87 mmol).

R_F = 0.80 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.9 Hz, 3H, CH_3), 1.26 – 1.42 (m, 16H, 8*CH_2), 1.85 (m, 2H, CH_2), 2.05 (q, ^3J = 6.9 Hz, 4H, 2*CH_2), 2.77 (m, 2H, CH_2), 3.40 (t, ^3J = 6.9 Hz, 2H, CH_2), 5.30 – 5.42 (m, 4H, 4*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.4 (CH_3), 22.9 (CH_2), 26.0 (CH_2), 27.5 (2*CH_2), 28.5 (CH_2), 29.1 (CH_2), 29.5 (CH_2), 29.7 (2*CH_2), 29.9 (CH_2), 31.9 (CH_2), 33.2 (CH_2), 34.3 (CH_2), 128.2 (CH), 128.4 (CH), 130.4 (CH), 130.5 (CH).

EI-MS (70eV): *m/z* (%) = 330 (13) [M^{+}_{81Br}], 328 (13) [M^{+}_{79Br}], 232 (2), 230 (3), 151 (4), 137 (11), 123 (16), 109 (33), 95 (65), 81 (85), 67 (100), 55 (66), 41 (75).

Synthesis of (6*Z*,9*Z*)-6,9-heptacosadiene (9)

A solution of **8** (0.617 g, 1.47 mmol) in 2 mL anhydr. tetrahydrofurane (THF) was cooled to -78°C and 10 mL of a solution of *n*-nonyl magnesium bromide, prepared from 0.057 g (2.4 mmol) Mg and 0.465 g (2.2 mmol) 1-bromononane in anhydr. THF, was added dropwise. A solution of Li_2CuCl_4 in THF (50µL, 0.005 mmol, 0.1M) was added and the temperature was maintained for 4h at -78°C, increased then to 0°C, and stirring was continued for 4h. Then, 10 mL of a saturated solution of NH_4Cl was added. The organic phase was separated and the aqueous phase was washed twice with diethyl ether. The combined organic phases were dried with $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to provide 0.077 g (0.20 mmol, 14%) of **9**.

R_F = 0.80 (pentane).

RI = 2670.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 – 0.91 (m, 6H, 2*CH_3), 1.26 – 1.39 (m, 36H, 18*CH_2), 2.02 – 2.08 (m, 4H, 2*CH_2), 2.78 (t, ^3J = 6.4 Hz, 2H, CH_2), 5.30 – 5.42 (m, 4H, 4*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (2*CH_3), 22.6 – 32.0 (21*CH_2), 128.0 (2*CH), 130.2 (2*CH).

EI-MS (70eV): *m/z* (%) = 376 (18) [M^+], 278 (3), 138 (13), 124 (23), 110 (44), 96 (76), 81 (84), 67 (100), 55 (72), 43 (93).

Synthesis of (*Z*)-3-nonenal (20)

A suspension of 10 g (60 mmol) PCC in 100 mL anhydr. CH_2Cl_2 was prepared, cooled to 0°C,

followed by the addition of a solution of 0.7 g (5 mmol) (*Z*)-3-nonen-1-ol in 30 mL anhydr. CH_2Cl_2 over a period of 5 min. At this temperature, the mixture was stirred for 3h. Then, the mixture was directly purified with silica gel and **20** was eluted with CH_2Cl_2. This compound was obtained as colourless liquid with a cucumber-like smell in 72% yield (0.5 g, 3.6 mmol).

RI = 1116.

δ_H [ppm](200MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.4 Hz, 3H, CH_3), 1.18 – 1.46 (m, 6H, 3*CH_2), 1.98 – 2.09 (m, 2H, CH_2), 3.19 (ddd, ^4J = 1.5 Hz , ^3J = 2.0 Hz, ^3J = 7.1 Hz, 2H, CH_2), 5.54 (ttd, ^4J = 1.4 Hz , ^3J = 7.1 Hz, ^3J = 10.9 Hz, 1H, CH), 5.70 (ttd, ^4J = 1.5 Hz , ^3J = 7.2 Hz, ^3J = 10.7 Hz, 1H, CH), 9.66 (t, ^3J = 2.0 Hz, 1H, CH).

δ_C [ppm](50MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.5 (CH_2), 27.6 (CH_2), 29.0 (CH_2), 31.4 (CH_2), 42.5 (CH_2), 117.9 (CH), 135.5 (CH), 199.7 (CH).

EI-MS (70eV): *m*/*z* (%) = 140 (0.2) [M^+], 122 (3), 111 (4), 96 (22), 84 (50), 69 (69), 55 (92), 41 (100).

Synthesis of docosyl benzoate (34)

This compound was synthesized according to the general procedure **A**.

Used amounts: 0.100 g (0.82 mmol) benzoic acid, 0.267 g (0.82 mmol) docosanol, 0.010 g (0.08 mmol) DMAP, 0.173 g (0.90 mmol) EDC and 5 mL anhydr. CH_2Cl_2, yield: 72% (0.59 mmol, 0.253g)

R_F = 0.83 (pentane/diethyl ether 5/1).

RI = 3273

δ_H [ppm](300MHz, $CDCl_3$, TMS): 0.86 – 0.90 (m, 3H, CH_3), 1.25 – 1.47 (m, 38H, 19*CH_2), 1.71 – 1.81 (m, 2H, CH_2), 4.31 (t, ^3J = 6.7 Hz, 2H, CH_2), 7.40 – 7.47 (m, 2H, 2*CH), 7.52 – 7.58 (m, 1H, CH), 8.02 – 8.06 (m, 2H, 2*CH).

δ_C [ppm](75 MHz, $CDCl_3$, TMS): 13.7 (CH_3), 22.3 – 31.5 (20*CH_2), 64.7 (CH_2), 127.9 (2*CH), 129.1 (2*CH), 130.1 (Cq) 132.3 (CH), 166.3 (Cq).

EI-MS (70eV): *m*/*z* (%) = 430 (0.9) [M^+], 308 (3), 280 (1), 123 (100), 105 (36), 97 (10), 83 (11), 77 (14), 69 (12), 57 (17), 43 (22).

Synthesis of 1-bromoheptane-7-ol (36)

This compound was synthesized according to the general procedure **B**.

Used amounts: 2g (10.25 mmol) 1,7-heptanediol, 10 mL 48% aqueous HBr-solution, 3 mL water; 4h reflux, 38% yield (1.122g, 3.90 mmol).

R_F = 0.22 (pentane/diethyl ether 1/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.31 – 1.49 (m, 6H, 3*CH_2), 1.54 – 1.61 (m, 2H, CH_2), 1.87 (m, 2H, CH_2), 3.41 (t, ^3J = 6.8 Hz, 2H, CH_2), 3.64 (t, ^3J = 6.6 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 25.5 (CH_2), 28.1 (CH_2), 28.5 (CH_2), 32.6 (CH_2), 32.7 (CH_2), 33.9 (CH_2), 62.9 (CH_2).

Synthesis of 2-(7-bromoheptyl-1-oxy)oxane (37)

This compound was synthesized according to the general procedure **C**.

Used amounts: 1.1 g (5.64 mmol) **36**, 0.474 g (5.64 mmol) 3,4-dihydro-2*H*-pyran, and 0.096 g (0.56 mmol) *p*-TsOH, 83% yield (1.301g, 4.68 mmol).

R_F = 0.43 (pentane/diethyl ether 5/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.30 – 1.90 (m, 16H, 8*CH_2), 3.36 – 3.42 (m, 3H, C<u>H</u>H`, $CH_2$), 3.47 – 3.55 (m, 1H, C<u>H</u>H`), 3.73 (td, ^2J = 9.6 Hz, ^3J = 6.8 Hz, 1H, CH<u>H</u>`), 3.84 – 3.90 (m, 1H, CH<u>H</u>`), 4.56 – 4.58 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 19.7 (CH_2), 25.5 (CH_2), 26.1 (CH_2), 28.1 (CH_2), 28.6 (CH_2), 29.6 (CH_2), 30.8 (CH_2), 32.8 (CH_2), 33.9 (CH_2), 62.3 (CH_2), 67.5 (CH_2), 98.9 (CH).

Synthesis of 2-(8-methyl-nonyl-1-oxy)oxane (38)

Compound **37** (0.6 g, 2.17 mmol) and 1.1 mL of a 2M solution of isopropyl magnesium chloride in THF were dissolved in 10 mL anhydr. THF, cooled to 0°C, and 0.2 mL (0.02 mmol) of a 0.1M solution of $Li_2[CuCl_4]$ were added [Tamura and Kochi, 1971]. The mixture was allowed to warm to rt and stirred for 24h. The mixture was quenched with 10 mL 2M aqueous HCl-solution and 10 mL diethyl ether were added. The organic phase was separated and the aqueous layer was washed twice with 10 mL of diethyl ether. The organic phases were combined and dried with $MgSO_4$. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 10/1) to furnish 0.346 g (1.43 mmol) **38** in 83% yield.

R_F = 0.74 (pentane/diethyl ether 5/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 (d, ^3J = 6.6 Hz, 6H, 2*CH_3), 1.12- 1.20 (m, 2H, CH_2), 1.27 – 1.39 (m, 8H, 4*CH_2), 1.46 – 1.63 (m, 7H, CH, 3*CH_2), 1.68 – 1.75 (m, 1H, C<u>H</u>H`), 1.79 – 1.87 (m, 1H, CH<u>H</u>`), 3.39 (td, ^3J = 6.7 Hz, ^2J = 9.4 Hz, 1H, C<u>H</u>H`), 3.47 – 3.54 (m, 1H, C<u>H</u>H`), 3.73 (td, ^3J = 6.9 Hz, ^2J = 9.6 Hz, 1H, CH<u>H</u>`), 3.85 – 3.90 (m, 1H, CH<u>H</u>`), 4.58 – 4.57 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 19.7 (CH_2), 22.6 (2*CH_3), 25.5 (CH_2), 26.2 (CH_2), 27.3 (CH_2), 28.0 (CH), 29.5 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 30.8 (CH_2), 39.0 (CH_2), 62.3 (CH_2), 67.7 (CH_2), 98.8 (CH).

Synthesis of 8-methyl-nonan-1-ol (39)

This compound was synthesized according to the general procedure **D**.

Used amounts: 0.346 (1.44 mmol) **38**, 0.025 g (0.14 mmol) *p*-TsOH, 5 mL methanol, yield 91% (0.188 g, 1.31 mmol).

R_F = 0.17 (pentane/diethyl ether 5/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 (d, ^3J = 6.7 Hz, 6H, 2*CH_3), 1.12 – 1.38 (m, 9H, OH, 4*CH_2), 1.47 – 1.60 (m, 3H, CH, CH_2), 3.66 (t, ^3J = 6.7 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 22.6 (2*CH_3), 25.7 (CH_2), 27.3 (CH_2), 28.0 (CH), 29.5 (CH_2), 29.9 (CH_2), 32.8 (CH_2), 39.0 (CH_2), 63.1 (CH_2).

EI-MS (70eV): *m*/*z* (%) = 140 (0.3), 125 (5), 112 (8), 97 (23), 83 (29), 69(79), 56 (100), 43 (60).

Synthesis of 8-methylnonanal (40)

A suspension of 0.107 g (0.5 mmol) PCC in 5 mL anhydr. CH_2Cl_2 was prepared followed by addition of 0.051g (0.33 mmol) **39**, dissolved in 1 mL anhydr. CH_2Cl_2 [Corey and Suggs, 1975]. The mixture was stirred for 24 h at rt and then filtered over silica gel (pentane/diethyl ether 5/1) to furnish 0.035 g (0.22 mmol) **40** in 67% yield.

R_F = 0.74 (pentane/diethyl ether 5/1).

RI = 1182.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 (d, ^3J = 6.6 Hz, 6H, 2*CH_3), 1.12 – 1.40 (m, 8H, 4*CH_2), 1.46 – 1.60 (m, 1H, CH), 1.61 – 1.70 (m, 2H, CH_2), 2.42 (dt, ^3J = 1.9 Hz, 7.4 Hz, 2H, CH_2), 9.76 (t, ^3J = 1.9 Hz, 1H, CH).

δ_C [ppm](100MHz, $CDCL_3$, TMS): 22.1 (CH_2), 22.6 (2*CH_3), 27.2 (CH_2), 27.9 (CH), 29.2 (CH_2), 29.6 (CH_2), 38.9 (CH_2), 43.9 (CH_2), 203.0 (CH).

EI-MS (70eV): *m*/*z* (%) = 138 (0.7), 128 (5), 123 (9), 112 (8), 110 (8), 95 (35), 82 (54), 69 (50), 57 (100), 43 (83).

Synthesis of tridecyl acetate (41)

This compound was synthesized according to the general procedure **E**.

Used amounts: 0.212 g (1.06 mmol) tridecanol, 0.110 g (0.1 mL, 1.4 mmol) acetyl chloride, and 0.110 g (0.11 mL, 1.4 mmol) pyridine, yield: 89% (0.228 g, 0.94 mmol).

R_F = 0.63 (pentane/diethyl ether 5/1).

RI = 1710.

δ_H [ppm](400MHz, $CDCL_3$, TMS): 0.88 (t, ^3J = 6.9 Hz, 3H, CH_3), 1.23 – 1.35 (m, 20H, 10*CH_2), 1.58 – 1.65 (m, 2H, CH_2), 2.04 (s, 3H, CH_3), 4.05 (t, ^3J = 6.8 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCL_3$, TMS): 14.1 (CH_3), 21.0 (CH_3), 22.7 (CH_2), 25.9 (CH_2), 28.6 (CH_2), 29.3 (2*CH_2), 29.5 (CH_2), 29.6 (3*CH_2), 29.7 (CH_2), 31.9 (CH_2), 64.6 (CH_2), 171.2 (C_q).

EI-MS (70eV): *m*/*z* (%) = 182 (5) [M-CH_3COOH]$^+$, 154 (8), 125 (8), 111 (14), 97 (31), 83 (41), 69 (43), 61 (41), 55 (49), 43 (100).

Synthesis of *n*-tetradecyl acetate (42)

This compound was synthesized according to the general procedure **E**.

Used amounts: 0.196 g (0.92 mmol) tetradecanol, 0.110 g (0.1 mL, 1.4 mmol) acetyl chloride, and 0.110 g (0.11 mL, 1.4 mmol) pyridine, yield: 89% (0.210 g, 0.82 mmol).

R_F = 0.63 (pentane/diethyl ether 5/1).

RI = 1808.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 6.8 Hz, 3H, CH_3), 1.22 – 1.35 (m, 22H, 11*CH_2), 1.58 – 1.65 (m, 2H, CH_2), 2.04 (s, 3H, CH_3), 4.05 (t, ^3J = 6.8 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (CH_3), 21.0 (CH_3), 22.7 (CH_2), 25.9 (CH_2), 28.6 (CH_2), 29.3 (2*CH_2), 29.5 (CH_2), 29.6 (3*CH_2), 29.7 (2*CH_2), 31.9 (CH_2), 64.6 (CH_2), 171.2 (C_q).

EI-MS (70eV): *m*/*z* (%) = 196 (4) [M-CH_3COOH]$^+$, 168 (5), 125 (8), 111 (15), 97 (29), 83 (43), 69 (43), 61 (35), 55 (48), 43 (100).

Synthesis of of (9*Z*,12*Z*)-9,12-octadecadienyl acetate (43)

This compound was synthesised according to the general procedure **E**.

Used amounts: 0.150 g (0.56 mmol) **7**, 0.062 g (56 µL, 0.78 mmol) acetyl chloride, and 0.062 g (56 µL, 0.78 mmol) pyridine, yield: 93% (0.170 g, 0.52 mmol).

R_F = 0.63 (pentane/diethyl ether 3/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.9 Hz, 3H, CH_3), 1.24 – 1.39 (m, 16H, 8*CH_2), 1.62 (quint, ^3J = 6.9 Hz, 2H, CH_2), 2.03 – 2.09 (m, 7H, 2*CH_2, CH_3), 2.76 – 2.79 (m, 2H, CH_2), 4.05 (t, ^3J = 6.8 Hz, 2H, CH_2), 5.29 – 5.42 (m, 4H, 4*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (CH_3), 21.0 (CH_3), 22.6 (CH_2), 25.6 (CH_2), 25.9 (CH_2), 27.2 (2*CH_2), 28.6 (CH_2), 29.2 (2*CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 31.5 (CH_2), 64.6 (CH_2), 127.9 (CH), 128.0 (CH), 130.1 (CH), 130.2 (CH), 171.2 (Cq).

EI-MS (70eV): *m*/*z* (%) = 308 (6) [M$^+$], 265 (2), 248 (8), 191 (2), 177 (3), 163 (5), 149 (9), 135 (20), 121 (30), 109 (23), 95 (56), 81 (79), 67 (100), 61 (8), 55 (62), 43 (96).

Synthesis of 8-heptadecyne (46)

A solution of 3 g (24.2 mmol) 1-nonyne in 60 mL anhydr. THF was prepared and cooled to -78°C

followed by a dropwise addition of 13.8 mL (22.1 mmol, 1.6M) *n*-BuLi solution in hexane [Buck and Chong, 2001]. The mixture was allowed to warm up to rt. A mixture of 0.3 g (2mmol) NaI and 3.86 g (20 mmol) 1-bromooctane, dissolved in 10 mL anhydr. THF, was added and the mixture was heated to reflux. After 40 h, the mixture was cooled to 0°C, a saturated aqueous solution of NH_4Cl (20 mL) was added, followed by dilution with H_2O (50 mL) and diethyl ether (40 mL). The organic phase was separated and the aqueous layer was washed twice 50 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to provide **46** in 14% yield (0.660 g, 2.80 mmol).

R_F = 0.65 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.87 – 0.90 (m, 6H, 2*CH_3), 1.28 – 1.55 (m, 22H, 11*CH_2), 2.12 – 2.15 (m, 4H, 2*CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (2*CH_3), 18.8 (2*CH_2), 22.7 (2*CH_2), 28.9 (3*CH_2), 29.2 (3*CH_2), 29.3 (CH_2), 31.8 (CH_2), 31.9 (CH_2), 80.0 (2*Cq).

EI-MS (70eV): *m/z* (%) = 236 (0.3) [M^+], 193 (3), 179 (4), 151 (2), 137 (11), 123 (23), 109 (35), 95 (74), 81 (100), 67 (87), 55 (58), 41 (54).

Synthesis of (*Z*)-8-heptadecene (47)

A solution of 0.060 g (2.4 mmol) **46** in 4 mL methanol was prepared and 0.007 g Lindlar-catalyst were added. Hydrogen from the mains system was sequentially added under atmospheric pressure and the progress of the reaction was controlled by GC. After 95 min, the conversion was complete and the crude product was filtered through silica gel (pentane) to furnish **47** quantitatively.

RI = 1678.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 – 0.90 (m, 6H, 2*CH_3), 1.22 – 1.35 (m, 22H, 11*CH_2), 1.96 – 2.06 (m, 4H, 2*CH_2), 5.31 – 5.39 (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 13.8 (2*CH_3), 22.4 (2*CH_2), 26.9 (2*CH_2), 28.9 (CH_2), 29.0 (3*CH_2), 29.2 (CH_2), 29.5 (2*CH_2), 31.6 (2*CH_2), 129.6 (2*CH).

EI-MS (70eV): *m/z* (%) = 238 (21) [M^+], 210 (2), 125 (15), 111 (36), 97 (71), 83 (85), 69 (89), 55 (100), 41 (76).

Synthesis of 2-octyne-1-ol (49)

A solution of 3 g (19.5 mmol) methyl 2-octynoate in 50 mL anhyr. diethyl ether was prepared and cooled to -78°C. A 1M DIBALH-solution (41 mL, 41 mmol) in hexane was added dropwise and the mxiture was stirred for 3h. The mixture was allowed to warm up to rt and was carefully quenched

with 20 mL methanol, resulting in the formation of a viscous heterogenous mixture. Addition of an appropriate amount of 5% aqueous sodium/potassium tartrate solution dissolved the gel and the organic phase was separated. The aqueous phase was washed twice with diethyl ether, the organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 2/1) to furnish 2.289 g (18.17 mmol) **49** in 93% yield.

R_F = 0.5 (pentane/diethyl ether 2/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.90 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.27 – 1.40 (m, 4H, 2*CH_2), 1.48 – 1.55 (m, 2H, CH_2), 1.93 (s, 1H, OH), 2.20 (tt, ^5J = 2.2 Hz, ^3J = 7.2 Hz, 2H, CH_2), 4.25 (t, ^5J = 2.2 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 13.9 (CH_3), 18.7 (CH_2), 22.1 (CH_2), 28.3 (CH_2), 31.0 (CH_2), 51.3 (CH_2), 78.3 (Cq), 86.5 (Cq).

Synthesis of 1-bromo-2-octyne (50)

A solution of 1.040 g (8.25 mmol) **49**, 0.869 g (10 mmol) LiBr, and 4.874 g (4.95 mL, 61.7 mmol) anhydr. pyridine in 15 mL anhydr. DMF was prepared, cooled to 0°C, and 1.530 g (1.03 mL, 13.44 mmol) methanesulfonyl chloride were added [Nilsson *et al.*, 1996]. The mixture was warmed to rt and the mixture was diluted with 20 mL diethyl ether. Furthermore, 20 mL H_2O were added and the organic phase was separated. The aqueous phase was washed twice with 20 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to furnish 0.540 g (2.87 mmol) **50** in 35% yield.

R_F = 0.75 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.90 (t, ^3J = 7.2 Hz, 3H, CH_3), 1.27 – 1.40 (m, 4H, 2*CH_2), 1.48 – 1.55 (m, 2H, CH_2), 2.23 (tt, ^5J = 2.3 Hz, ^3J = 7.2 Hz, 2H, CH_2), 4.14 (t, ^5J = 2.3 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 13.6 (CH_3), 18.5 (CH_2), 21.8 (CH_2), 27.7 (CH_2), 30.7 (CH_2), 30.9 (CH_2), 74.6 (Cq), 87.4 (Cq).

Synthesis of 6,9-heptadecadiyne (51)

A solution of 0.459 g (3.70 mmol) 1-nonyne **44** in 5 mL anhydr. THF was added to a freshly prepared solution of ethylmagnesium bromide in 10 mL anhydr. THF (0.120 g, 5 mmol Mg; 0.600 g, 0.41 mL, 5.5 mmol ethyl bromide) and the mixture was heated for 1.5 h at 45°C [Millar and Underhill, 1986]. The mixture was cooled to -10°C, 0.030 g (0.16 mmol)

Cu(I)I were added, and the mixture was stirred for 20 min. A solution of 0.700 g (3.70 mmol) **50** in 10 mL anhydr. THF was added dropwise and the mixture was allowed to warm to rt. The mixture was stirred overnight followed by quenching with H_2O (10 mL) and a saturated solution of NH_4Cl (10 mL). Pentane (20 mL) was added and the turbid organic phase was separated. The aqueous phase was washed twice with pentane (20 mL). The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to furnish 0.815 g (3.52 mmol) **51** in 95% yield.

R_F = 0.5 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 – 0.90 (m, 6H, 2*CH_3), 1.23 – 1.44 (m, 16H, 8*CH_2), 1.45 – 1.55 (m, 4H, 2*CH_2), 2.15 (tt, ^5J = 2.4 Hz, ^3J = 7.2 Hz, 4H, 2*CH_2), 3.12 (quint, ^5J = 2.4 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 9.7 (CH_2), 14.0 (CH_3), 14.1 (CH_3), 18.7 (2*CH_2), 22.2 (CH_2), 22.6 (CH_2), 28.4 (CH_2), 28.8 (2*CH_2), 28.9 (CH_2), 31.1 (CH_2), 31.8 (CH_2), 74.5 (2*Cq), 80.5 (2*Cq).

EI-MS (70eV): *m/z* (%) = 217 (1), 203 (2), 189 (5), 161 (8), 147 (9), 133 (20), 119 (29), 105 (57), 91 (100), 79 (37), 67 (36), 55 (26), 41 (54).

Synthesis of (6*Z*,9*Z*)-6,9-heptadecadiene 52 [Harada *et al.*, 1995]

Compound **51** (0.432 g, 1.86 mmol) was dissolved in 10 mL anhydr. diethyl ether and a solution of 2.641g (9.3 mmol) $Ti(OiPr)_4$ in 20 mL diethyl ether was added in one portion. This mixture was cooled to -78°C and a freshly prepared solution of isopropylmagnesium bromide (0.583 g, 24 mmol Mg; 2.95 g, 24 mmol 2-bromopropane) in 15 mL diethyl ether was added dropwise. The colour of the solution became yellow and the mixture was allowed to warm up to -30°C over a period of 2h. During this time, the colour of the mixture changed to brown. The mixture was recooled to -78°C, H_2O (20 mL) was added, the mixture was allowed to warm to rt, and stirring was continued overnight. The reaction was quenched by the addition of 20 mL of a saturated solution of NH_4Cl and the organic layer was separated. The aqueous phase was washed twice with 50 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to furnish 0.022 g (0.09 mmol) **52** in 5% yield.

R_F = 0.83 (pentane).

RI = 1671.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 – 0.90 (m, 6H, 2*CH_3), 1.21 – 1.39 (m, 16H, 8*CH_2), 2.03 – 2.08 (m, 4H, 2*CH_2), 2.78 (t, ^3J = 6.4 Hz, 2H, CH_2), 5.30 – 5.42 (m, 4H, 4*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (2*CH_3), 22.6 (CH_2), 22.7 (CH_2), 25.7 (CH_2), 27.2 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 31.5 (CH_2), 31.9 (CH_2), 128.0 (2*CH), 130.2 (2*CH).

EI-MS (70eV): *m/z* (%) = 236 (16) [M^+], 166 (1), 152 (2), 138 (10), 124 (13), 110 (28), 95 (52), 81 (77), 67 (100), 55 (52), 41 (58).

Synthesis of (*Z*)-1-iodo-non-3-ene (54)

A suspension of 8.20 g (31.29 mmol) PPh_3 and 2.13 g (31.29 mmol) imidazol in a solvent mixture (70 mL) of anhydr. acetonitrile and anhydr. diethyl ether in a ratio of 1:3 was prepared and cooled to 0°C. Iodine (7.95 g, 31.29 mmol) was added in portions [Pohnert and Boland, 2000]. The mixture was stirred for 10 min at rt, was then recooled to 0°C, followed by dropwise addition of a solution of 2 g (14.08 mmol) (*Z*)-3-nonen-1-ol **53** in 5 mL anhydr. diethyl ether. The mixture was allowed to warm up to rt and stirring was continued overnight. An aqueous saturated solution of $NaHCO_3$ (30 mL) was added and the organic phase was separated. The aqueous phase was washed twice with 30 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 5/1) to provide 3.02 g (11.98 mmol) **54** in 85% yield.

R_F = 0.5 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.8 Hz, 3H, CH_3), 1.23 – 1.41 (m, 6H, 3*CH_2), 1.98 – 2.05 (m, 2H, CH_2), 2.60 – 2.66 (m, 2H, CH_2), 3.13 (t, ^3J = 6.8 Hz, 2H, CH_2), 5.27 – 5.36 (m, 1H, CH), 5.48 – 5.58 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 5.5 (CH_2), 14.0 (CH_3), 22.5 (CH_2), 27.4 (CH_2), 29.1 (CH_2), 31.5 (2*CH_2), 127.7 (CH), 132.7 (CH).

EI-MS (70eV): *m/z* (%) = 252 (0.6) [M^+], 209 (0.1), 196 (1), 181 (0.7), 167 (0.8), 155 (4), 141 (2), 125 (9), 83 (56), 69 (100), 55 (73), 41 (47).

Synthesis of ((*Z*)-3-nonenyl)triphenylphosphonium iodide (55)

PPh_3 (3.14 g, 11.98 mmol) were dissolved in 55 mL anhydr. acetonitrile followed by addition of 3.02 g (11.98 mmol) **54** in 10 mL anhydr. acetonitrile [Pohnert and Boland, 2000]. The mixture was heated to reflux for 4h. Then, the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel. First, excess PPh_3 was removed by elution with pentane followed by elution of the Wittig-salt with CH_2Cl_2 to provide **55** in 45% yield (2.77 g, 5.39 mmol).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.86 (t, ^3J = 7.0 Hz, 3H, CH_3), 1.15 – 1.32 (m, 6H, 3*CH_2),

1.85 – 1.90 (m, 2H, CH_2), 2.38 – 2.46 (m, 2H, CH_2), 3.43 – 3.52 (m, 2H, CH_2), 5.43 – 5.53 (m, 2H, 2*CH), 7.24 – 7.34 (m, 3H, 3*CH), 7.74 - 7.92 (m, 12H, 12*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.4 (CH_3), 21.2 (CH_2, $J_{P,C}$ = 3.5 Hz), 23.0 (CH_2, $J_{P,C}$ = 48.9 Hz), 23.5 (CH_2), 28.2 (CH_2), 30.1 (CH_2), 32.6 (CH_2), 119.9 (3*Cq, $J_{P,C}$ = 86.3 Hz), 127.1 (CH, $J_{P,C}$ = 15.6 Hz), 129.6 (CH, $J_{P,C}$ = 6.9 Hz), 131.6 (6*CH, $J_{P,C}$ = 12.6 Hz), 131.9 (6*CH, $J_{P,C}$ = 10.1 Hz), 136.1 (3*CH, $J_{P,C}$ = 3.0 Hz).

δ_P [ppm](162MHz, $CDCl_3$, TMS): 24.9 (s).

Synthesis of (6*Z*,9*Z*)-6,9-heptadecadiene (52)

A solution of 0.59 g (1.15 mmol) **55** in 25 mL anhydr. dimethoxyethane (DME) was cooled to -78° C and 1.26 mL (2.53 mmol) of a 2M NaHMDS-solution in THF were added dropwise [Pohnert and Boland, 2000]. The mixture was allowed to warm up to rt and stirring was continued for 30 min. Then, the mixture was recooled to -78°C and a solution of 0.150 g (1.15 mmol) octanal in 3 mL DME was added. The cooling bath was removed and stirring was continued for overnight at rt. The mixture was quenched with 10 mL of a 2M aqueous HCl-solution, 10 mL diethyl ether were added, and the organic phase was separated. The aqueous phase was washed twice with 20 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to provide 0.22 g (0.93 mmol) **52** in 81% yield. The product was obtained in a *ZZ*/*ZE*-ratio of 94:6.

Synthesis of 2-methoxyhenicosane (58)

a) Preparation of 2-henicosanol **57**:

This compound was synthesized according to the general procedure **I**.

Used amounts: 0.123 g (0.42 mmol) eicosanal, 0.42 mL (1.26 mmol) 3M methylmagnesium chloride in THF, yield: 95% (0.125g, 0.40 mmol).

b) Preparation of **58**:

A suspension of 0.026 g (0.86 mmol) sodium hydride (80% in mineral oil) in 5 mL anhydr. THF was prepared, stirred for 30 min at rt, followed by the addition of a solution of 0.125 g (0.40 mmol) **57** in 3 mL anhydr. THF and two spatula tips of tetrabutylammonium iodide. The mixture was heated to reflux for 3.5h and stirring at rt was continued overnight. The mixture was quenched with 5 mL 2M aqueous HCl-solution, 10 mL diethyl ether were added, and the organic phase was separated. The aqueous phase was washed twice with 10 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude

product was purified by column chromatography on silica gel (pentane/diethyl ether 20/1) to provide **58** (0.124g, 0.38 mmol) in 95% yield.

R_F = 0.45 (pentane/diethyl ether 20/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 6.8 Hz, 3H, CH_3), 1.12 (d, ^3J = 6.1 Hz, 3H, CH_3), 1.26 – 1.32 (m, 36H, 18*CH_2), 3.25 – 3.33 (m, 4H, CH, CH_3).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (CH_3), 19.0 (CH_3), 22.7 – 36.4 (18*CH_2), 55.9 (CH_3), 76.9 (CH).

EI-MS (70eV): *m/z* (%) = 311 (1) $[M\text{-}CH_3]^+$, 294 (1), 266 (0.3), 111 (2), 97 (4), 83 (4), 69 (5), 59 (100), 43 (12).

Synthesis of 2-(prop-2-ynoxy)oxane (79)

This compound was synthesized according to the general procedure **C**.

Used amounts: 2.889 g (3.0 mL, 53.6 mmol) **78**, 30 mL anhydr. diethyl ether, 4.5 g (53.6 mmol) 3,4-dihydro-2*H*-pyran in 10 mL anhydr. diethyl ether, 1g (5.36 mmol) *p*-TsOH, yield: 71% (5.3 g, 37.9 mmol).

The solution of 3,4-dihydro-2*H*-pyran was added dropwise at 0°C.

R_F = 0.7 (pentane/diethyl ether 2/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.52 – 1.88 (m, 6H, 3*CH_2), 2.44 (t, ^4J = 2.5 Hz, 1H, CH), 3.51 – 3.56 (m, 1H, CH<u>H</u>`), 3.81 – 3.86 (m, 1H, C<u>H</u>H`), 4.26 (ddd, ^4J = 2.4 Hz, ^2J = 15.7 Hz, 2H, CH_2), 4.82 (t, ^3J = 3.4 Hz, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 19.1 (CH_2), 25.5 (CH_2), 30.3 (CH_2), 54.1 (CH_2), 62.1 (CH_2), 74.2 (CH), 79.9 (Cq), 96.9 (CH).

EI-MS (70eV): *m/z* (%) = 139 (12) [M^+-1], 101 (6), 85 (100), 82 (14), 67 (13), 56 (49), 41 (51), 39 (62).

Synthesis of 2-(octa-2,5-diynoxy)oxane (81)

A suspension of 2.040 g (13.60 mmol) NaI, 1.295 g (6.80 mmol) CuI, and 1.880 g (13.60 mmol) K_2CO_3 in 10 mL anhydr. DMF was prepared [Lapitskaya *et al.*, 1993]. A solution of 0.952 g (6.80 mmol) **79** in 5 mL DMF was added followed by a solution of 1 g (6.80 mmol) 1-bromo-2-pentyne in 5 mL DMF. The mixture was stirred for 90h at rt. The mixture was quenched with 10 mL H_2O and 10 mL saturated NH_4Cl-solution. The organic phase was separated after the addition of 10 mL diethyl ether. The aqueous phase was washed twice with 10 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel

(pentane/diethyl ether 10/1) to furnish **81** (1.160 g, 5.63 mmol) in 83% yield.

R_F = 0.13 (pentane/diethyl ether 10/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.12 (t, ^3J = 7.5 Hz, 3H, CH_3), 1.52 – 1.87 (m, 6H, 3*CH_2), 2.17 (tq, ^5J = 2.4 Hz, ^3J = 7.5 Hz, 2H, CH_2), 3.19 (quint, ^5J = 2.3 Hz, 2H, CH_2), 3.50 – 3.55 (m, 1H, C<u>H</u>H`), 3.81 – 3.86 (m, 1H, CH<u>H</u>`), 4.25 (tdd, ^2J = 15.4 Hz, ^5J = 2.2 Hz, 2H, CH_2), 4.80 (t, ^3J = 3.4 Hz, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 9.8 (CH_2), 12.2 (CH_2), 13.7 (CH_3), 19.0 (CH_2), 25.3 (CH_2), 30.1 (CH_2), 54.4 (CH_2), 61.8 (CH_2), 72.7 (Cq), 75.9 (Cq), 80.8 (Cq), 82.2 (Cq), 96.7 (CH).

EI-MS (70eV): *m/z* (%) = 205 (3) [M^+ -1], 191 (0.6), 177 (1), 163 (4), 149 (4), 135 (5), 121 (6), 117 (8), 111 (9), 103 (52), 101 (26), 91 (73), 85 (100), 77 (97), 67 (23), 55 (35), 41 (48).

Synthesis of 1-bromo-2,5-octadiyne (82)

A solution of 0.838 g (3.20 mmol) PPh_3 in 30 mL anhydr. CH_2Cl_2 was prepared, cooled to 0°C, followed by the addition 0.512 g (0.164 mL, 3.20 mmol) bromine [Sonnet, 1976]. A heterogenous yellow mixture was formed and additional PPh_3 was added to remove excess bromine in this mixture. A solution of 0.600 g (2.91 mmol) **81** in 5 mL anhydr. CH_2Cl_2 was added and the mixture was allowed to warm up to rt. Stirring at rt was continued overnight and then 20 mL H_2O were added. The organic phase was separated and the aqueous phase was extracted twice with 20 mL CH_2Cl_2. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to provide **82** (0.447 g, 2.43 mmol) in 84% yield.

R_F = 0.08 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.12 (t, ^3J = 7.5 Hz, 3H, CH_3), 2.17 (tq, ^5J = 2.4 Hz, ^3J = 7.5 Hz, 2H, CH_2), 3.21 (quint, ^5J = 2.4 Hz, 2H, CH_2), 3.92 (t, ^5J = 2.3 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 10.0 (CH_2), 12.3 (CH_2), 13.7 (CH_3), 14.8 (CH_2), 72.1 (Cq), 75.2 (Cq), 82.0 (Cq), 82.6 (Cq).

EI-MS (70eV): *m/z* (%) = 186 (16) [M^+_{81Br}], 184 (16) [M^+_{79Br}], 171 (3) [M-CH_3]$^+$, 169 (3) [M-CH_3]$^+$, 105 (67), 89 (17), 77 (100), 63 (43), 51 (48), 39 (30).

Synthesis of 2-(9-decynoxy)oxane (86)

a) Preparation of **85**:

This compound was synthesized according to the general procedure **C**.

1.818 g (9.05 mmol) **84**, 0.731 g (9.05 mmol) 3,4-dihydro-2*H*-pyran, 0.165 g (0.90 mmol) *p*-TsOH,

yield: 94% (2.024 g, 8,51 mmol); determined by TLC: R_F = 0.25 (pentane/diethyl ether 20/1).

EI-MS (70eV): *m/z* (%) = 321 (2) [M^+-1 (^{81}Br)], 319 (3) [M^+-1 (^{79}Br)], 101 (10), 85 (100), 69 (13), 55 (30), 41 (34).

Crude **85** was used without further purification for the conversion to **86**.

A suspension of 0.542 g (5.89 mmol) lithium acetylid-ethylenediamine-complex in 5 mL anhydr. dimethylsulfoxide (DMSO) was prepared, cooled to 0°C, followed by the addition of 1.273 g (4.35 mmol) **85**. The mixture was warmed to rt and stirred overnight. The mixture was poured into 50 mL ice water and 50 mL diethyl ether were added. The organic phase was separated and the aqueous phase was washed twice with 50 mL diethyl ether. The combined organic phases were washed three times with 20 mL H_2O, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 20/1) to provide **86** (0.742 g, 3.12 mmol) in 72% yield.

R_F = 0.21 (pentane/diethyl ether 20/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.26 (m, 18H, 9*CH_2), 1.93 (t, ^4J = 2.7 Hz, 1H, CH), 2.18 (dt, ^4J = 2.6 Hz, ^3J = 7.0 Hz, 2H, CH_2), 3.38 (td, ^3J = 6.7 Hz, ^2J = 9.6 Hz, 1H, C<u>H</u>H`), 3.48 – 3.52 (m, 1H, C<u>H</u>H`), 3.73 (td, ^3J = 6.9 Hz, ^2J = 9.6 Hz, 1H, CH<u>H</u>`), 3.84 – 3.90 (m, 1H, CH<u>H</u>`), 4.56 – 4.58 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 18.4 (CH_2), 19.7 (CH_2), 25.5 (CH_2), 26.2 (CH_2), 28.4 (CH_2), 28.7 (CH_2), 29.0 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 30.8 (CH_2), 62.3 (CH_2), 67.6 (CH_2), 68.0 (CH), 84.7 (Cq), 98.8 (CH).

EI-MS (70eV): *m/z* (%) = 237 (2) [M^+-1], 101 (27), 85 (100), 81 (20), 79 (10), 67 (22), 55 (28), 41 (38).

Synthesis of 2-(octadeca-9,12,15-triynoxy)oxane (87)

A suspension of 0.804 g (5.36 mmol) NaI, 0.400 g (2.10 mmol) CuI, and 0.739 g (5.36 mmol) K_2CO_3 in 4 mL anhydr. DMF was prepared [Lapitskaya *et al.*, 1993]. A solution of 0.638 g (2.68 mmol) **86** in 3 mL DMF was added, followed by a solution of 0.396 g (2.14 mmol) **82** in 3 mL DMF. After the addition of **82** was finished, evolution of heat was immediately noticed and stirring was continued for 5h. Then, again 0.110 g (0.58 mmol) CuI and 0.100g (0.54 mmol) **82** in 1 mL DMF were added, heat evolved and stirring was continued for 48h. The reaction was worked up analogously to the formation of **81**. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 20/1) to provide **87** (0.230 g, 0.84 mmol) in 31% yield.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.12 (t, ^3J = 7.5 Hz, 3H, CH_3), 1.25 – 1.87 (m, 18H, 9*CH_2),

2.12 – 2.20 (m, 4H, 2*CH_2), 3.13 (br. s, 4H, 2*CH_2), 3.38 (td, ^3J = 6.7 Hz, ^2J = 9.6 Hz, 1H, C<u>H</u>H`), 3.47 – 3.53 (m, 1H, C<u>H</u>H`), 3.73 (td, ^3J = 6.9 Hz, ^2J = 9.5 Hz, 1H, CH<u>H</u>`), 3.84 – 3.90 (m, 1H, CH<u>H</u>`), 4.57 – 4.59 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 9.7 (CH_2), 9.8 (CH_2), 12.4 (CH_2), 13.8 (CH_3), 18.7 (CH_2), 19.7 (CH_2), 25.5 (CH_2), 26.2 (CH_2), 28.7 (CH_2), 28.8 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 30.8 (CH_2), 62.3 (CH_2), 67.7 (CH_2), 73.2 (Cq), 73.8 (Cq), 74.8 (Cq), 74.9 (Cq), 80.8 (Cq), 82.1 (Cq), 98.8 (CH).

Synthesis of (9*Z*,12*Z*,15*Z*)-2-(9,10,12,13,15,16)-hexadeuterooctadeca-9,12,15-trienoxy)oxane (88)

A freshly prepared solution of isopropylmagnesium bromide, prepared from 2.46 g (1.88 mL, 20 mmol) isopropyl bromide and 0.475 g (20 mmol) Mg in 10 mL anhydr. diethyl ether, was added to a -78°C cooled solution of 0.325 g (0.96 mmol) **87** and 2.182 g (8 mmol) $Ti(OiPr)_4$ in 20 mL anhydr. diethyl ether [Hungerford and Kitching, 1998]. The mixture was warmed to -30°C and stirring was continued for 2h. The mixture was recooled to -78°C and 5 mL D_2O were added in one portion and the mixture was warmed to rt and stirred overnight. The mixture was quenched with 10 mL 10% aqueous HCl-solution and the organic phase was separated. The aqueous phase was washed twice with 20 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The product was formed only in traces with a label of four deuterium atoms in average randomly distributed in the triene system.

R_F = 0.63 (pentane/diethyl ether 10/1).

EI-MS (70eV): *m*/*z* (%) = 351 (0.9) [M^+-1], 296 (2), 294 (2), 139 (4), 125 (3), 112 (5), 110 (6), 101 (5), 97 (10), 85 (100), 82 (19), 69 (10), 67 (11), 56 (20), 41 (37).

Synthesis of (9*Z*,12*Z*,15*Z*)-9,10,12,13,15,16-hexadeuterooctadeca-9,12,15-trien-1-ol (89)

Compound **88** was deprotected by stirring 0.046 g of a fraction, containing 27% of **88**, with 0.010 g (0.05 mmol) *p*-TsOH in 5 mL methanol for 3.5h. Work-up according to the general procedure **D** provided 0.020 g of a fraction containing **89** in 39%.

R_F = 0.13 (pentane/diethyl ether 10/1).

EI-MS (70eV): *m*/*z* (%) = 268 (2) [M^+], 212 (3), 211 (3), 210 (4), 139 (5), 125 (7), 112 (15), 111 (21), 110 (29), 97 (36), 81 (100), 69 (53), 55 (79), 41 (88).

Synthesis of (9*Z*,12*Z*,15*Z*)-9,10,12,13,15,16-hexadeuterooctadeca-9,12,15-trienoic acid (90)

The product fraction of the synthesis of **89** was dissolved in 3 mL DMF and 0.150 g (0.40 mmol) pyrdinium dichromate (PDC) were added in one portion. The progress of the reaction was controlled by TLC. The reaction was not finished after 24h and therefore an additional amount of PDC (0.050 g, 0.13 mmol) was added and stirring was continued for further 24h. The crude mixture was purified by column chromatography on silica gel (pentane/diethyl ether 3/1) to furnish 0.002 g of pure (GC!) **90**.

R_F = 0.25 (pentane/diethyl ether 3/1).

EI-MS (70eV): *m*/*z* (%) = 354 (3) [M^+], 339 (47), 264 (15), 129 (49), 117 (41), 112 (2), 111 (5), 110 (10), 96 (22), 95 (24), 81 (38), 75 (94), 73 (100), 67 (50), 55 (48), 41 (34).

Synthesis of methyl (9*Z*,12*Z*,15*Z*)-2,2-dideuterooctadeca-9,12,15-trienoate (92)

Sodium (1.2 g, 52.2 mmol) were dissolved in 50 mL [D_1]-methanol and a solution of 1 g (3.42 mmol) methyl α-linolenoate in 8 mL [D_1]-methanol was added. The mixture was heated to reflux for 4h, diluted with 30 mL diethyl ether, followed by the addition of 80 mL 2M aqueous HCl. The organic phase was separated and the aqueous phase was washed twice with 30 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. A product mixture (0.993g) of **92** and the corresponding free acid was obtained and 95% of this mixture contained two deuterium atoms.

R_F = 0.83 (pentane/diethyl ether 2/1) methyl ester; R_F = 0.51 (pentane/diethyl ether 2/1) free acid.

EI-MS (70eV): *m*/*z* (%) = 294 (2) [M^+], 263 (2), 238 (2), 163 (3), 149 (9), 135 (11), 121 (14), 108 (30), 95 (46), 79 (100), 67 (66), 55 (41), 41 (54).

Synthesis of (9*Z*,12*Z*,15*Z*)-1,1,2,2-tetradeuterooctadeca-9,12,15-trien-1-ol (93)

This compound was synthesized according to the general procedure **F**.

Used amounts: 0.904 g (3.07 mmol) **92**, 0.225 g (5.37 mmol) lithium aluminium deuteride, 20 mL anhydr. diethyl ether, yield: 89% (0.726 g, 2.73 mmol).

R_F = 0.26 (pentane/diethyl ether 2/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.98 (t, ^3J = 7.6 Hz, 3H, CH_3), 1.25 – 1.40 (m, 10H, 5*CH_2), 2.03 – 2.12 (m, 4H, 2*CH_2), 2.79 – 2.82 (m, 4H, 2*CH_2), 5.28 – 5.43 (m, 6H, 6*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.3 (CH_3), 20.5 (CH_2), 25.5 (2*CH_2), 25.6 (CH_2), 27.2 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 127.1 (CH), 127.7 (CH), 128.3 (2*CH), 130.3 (CH), 131.9 (CH).

EI-MS (70eV): *m*/*z* (%) = 268 (1) [M^+], 212 (3), 149 (3), 135 (6), 121 (9), 108 (32), 95 (31),

93 (41), 79 (100), 67 (60), 55 (31), 41 (49).

Synthesis of (9*Z*,12*Z*,15*Z*)-1,1,2,2-tetradeutero-1-iodo-octadeca-9,12,15-triene (94)

A solution of 2.26 g (8.62 mmol) PPh_3 in 30 mL anhydr. THF was prepared, cooled to 0°C, followed by the addition of 3.51 mL (3.33 g, 7.65 mmol) diethyl azodicarboxylate (DEAD) [Manna *et al.*, 1985]. This mixture was stirred for 20 min, 2.10 g (15.62 mmol) LiI were added, and stirring was continued until the LiI was completely dissolved. A solution of 0.832 g (3.12 mmol) **93** in 5 mL anhydr. THF was added and the mixture was stirred overnight at rt. The mixture was quenched with 50 mL H_2O, 30 mL diethyl ether were added, and the organic phase was separated. The aqueous phase was washed four times with 20 mL diethyl ether. The organic phases were combined, washed with an aqueous saturated NaCl solution, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to furnish 0.806 g (2.14 mmol) **94** in 69% yield.

R_F = 0.5 (pentane).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.98 (t, ^3J = 7.5 Hz, 3H, CH_3), 1.26 – 1.35 (m, 10H, 5*CH_2), 2.02 – 2.13 (m, 4H, 2*CH_2), 2.81 (t, ^3J = 5.7 Hz, 4H, 2*CH_2), 5.27 – 5.42 (m, 6H, 6*CH).

δ_C [ppm](100MHz, $CDCL_3$, TMS): 14.3 (CH_3), 20.6 (CH_2), 25.5 (CH_2), 25.6 (CH_2), 27.2 (CH_2), 28.5 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.6 (CH_2), 30.3 (CH_2), 127.1 (CH), 127.7 (CH), 128.3 (2*CH), 130.3 (CH), 132.0 (CH).

EI-MS (70eV): *m*/*z* (%) = 378 (1) [M^+], 322 (11), 135 (8), 121 (11), 108 (59), 93 (40), 79 (100), 67 (61), 55 (27), 41 (38).

Synthesis of (9*Z*,12*Z*,15*Z*)-1,1,2,2-tetradeuterooctadeca-9,12,15-trien-1-carbonitrile (95)

Compound **94** (0.215 g, 0.57 mmol) was dissolved in 5 mL anhydr. DMSO and a solution of 0.178 g (1.14 mmol) tetraethylammonium cyanide in 20 mL DMSO was added. The progress of the reaction was controlled by TLC and after 1h the educt was completely consumed. The reaction was quenched with 20 mL H_2O and 20 mL diethyl ether were added. The organic phase was separated and the aqueous phase was washed twice with 20 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 10/1) to furnish 0.140 g (0.51 mmol) **95** in 89% yield.

R_F = 0.25 (pentane/diethyl ether 10/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.98 (t, ^3J = 7.6 Hz, 3H, CH_3), 1.31 – 1.43 (m, 10H, 5*CH_2), 2.03 – 2.12 (m, 4H, 2*CH_2), 2.79 – 2.82 (m, 4H, 2*CH_2), 5.28 – 5.43 (m, 6H, 6*CH).

δ_C [ppm](100MHz, $CDCL_3$, TMS): 14.3 (CH_3), 20.5 (CH_2), 25.5 (CH_2), 25.6 (CH_2), 27.2 (CH_2), 28.4 (CH_2), 28.7 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.5 (CH_2), 119.8 (Cq), 127.1 (CH), 127.8 (CH), 128.2 (CH), 128.3 (CH), 130.2 (CH), 131.9 (CH).

EI-MS (70eV): *m*/*z* (%) = 277 (2) [M^+], 248 (2), 234 (1), 221 (2), 208 (7), 149 (4), 135 (9), 121 (12), 108 (20), 95 (49), 79 (100), 67 (71), 55 (42), 41 (63).

Synthesis of (10*Z*,13*Z*,16*Z*)-2,2,3,3-tetradeuterononadeca-10,13,16-trienal (96)

This compound was synthesized according to the general procedure **H**.

Used amounts: 0.070 g (0.25 mmol) **95** dissolved in 5 mL anhydr. toluene, 0.34 mL (0.34 mmol) 1M DIBALH-solution in toluene, reaction time 1.5h, 5 mL H_2O, 2 pipettes 2.5% aqueous H_2SO_4-solution.

The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 10/1) to furnish 0.036 g (0.13 mmol) **96** in 50% yield.

R_F = 0.38 (pentane/diethyl ether 10/1).

EI-MS (70eV): *m*/*z* (%) = 280 (3) [M^+], 224 (4), 211 (2), 149 (5), 135 (12), 121 (15), 108 (37), 93 (51), 79 (100), 67 (68), 55 (29), 41 (49).

Synthesis of (10*Z*,13*Z*,16*Z*)-2,2,3,3-tetradeuterononadeca-10,13,16-trienoic acid (98)

a) Preparation of [D_4]-2,2,3,3-(10*Z*,13*Z*,16*Z*)-10,13,16-nonadecatrienol **97**:

This compound was synthesized according to the general procedure **F**.

Used amounts: 21 mg (0.075 mmol) **96**, 5.3 mg (0.13 mmol) LAH, yield: 84% (18 mg, 0.063 mmol) **97**.

R_F = 0.30 (pentane/diethyl ether 2/1).

EI-MS (70eV): *m*/*z* (%) = 282 (3) [M^+], 226 (4), 163 (1), 149 (5), 135 (8), 121 (13), 108 (43), 93 (41), 79 (100), 67 (60), 55 (29), 41 (41).

b) Preparation of **98**:

A solution of 18 mg (0.063 mmol) **97** in 3 mL DMF was prepared and 0.150g (0.40 mmol) PDC were added. After 24h the educt was completely consumed and 3 mL H_2O were added. Furthermore, the mixture was diluted with 3 mL diethyl ether and the organic phase was separated with a 10 mL-syringe. The aqueous phase was washed twice with 3 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 3/1) to furnish 5.6 mg (0.019 mmol) **98** in 30% yield.

R_F = 0.25 (pentane/diethyl ether 3/1).

EI-MS of the TMS-derivative of **98** (70eV): *m*/*z* (%) = 368 (2) [M^+], 353 (12), 312 (1), 132 (21), 119 (22), 108 (43), 95 (59), 93 (43), 79 (95), 76 (96), 73 (100), 67 (57), 55 (30), 41 (40).

Synthesis of (10*Z*,13*Z*,16*Z*)-2,2,3,3-tetradeuterononadeca-10,13,16-trien-1-carbonitrile (99)

This compound was synthesized according to the general procedure **G**.

Used amounts: 0.14 mL (0.108 g, 2.65 mmol) acetonitrile, 1.66 mL (2.65 mmol) of a 1.6M *n*-BuLi-solution in hexane, 0.400 g (1.06 mmol) **94**, yield: 61% (0.188 g, 0.64 mmol).

R_F = 0.25 (pentane/diethyl ether 10/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.98 (t, ^3J = 7.5 Hz, 3H, CH_3), 1.26 - 1.41 (m, 10H, 5*CH_2), 2.03 – 2.17 (m, 4H, 2*CH_2), 2.38 (s, 2H, CH_2), 2.81 (t, ^3J = 5.7 Hz, 4H, 2*CH_2), 5.27 – 5.42 (m, 6H, 6*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.3 (CH_3), 20.5 (CH_2), 25.5 (CH_2), 25.6 (CH_2), 27.2 (CH_2), 28.5 (CH_2), 28.7 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 119.8 (Cq), 127.1 (CH), 127.7 (CH), 128.2 (CH), 128.3 (CH), 130.3 (CH), 131.9 (CH).

EI-MS (70eV): *m*/*z* (%) = 291 (2) [M^+], 262 (2), 248 (2), 235 (2), 222 (6), 180 (2), 166 (3), 149 (5), 135 (9), 121 (12), 108 (24), 95 (53), 79 (100), 67 (73), 55 (41), 41 (61).

Synthesis of (11*Z*,14*Z*,17*Z*)-3,3,4,4-tetradeuteroeicosa-11,14,17-trienal (100)

This compound was synthesized according to the general procedure **H**.

Used amounts: 0.090 g (0.25 mmol) **99** dissolved in 5 mL anhydr. toluene, 0.42 mL (0.42 mmol) 1M DIBALH-solution in toluene, reaction time 1.5h, 5 mL H_2O, 2 pipettes 2.5% aqueous H_2SO_4-solution.

The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 10/1) to furnish 0.050 g (0.17 mmol) **100** in 54% yield.

R_F = 0.38 (pentane/diethyl ether 10/1).

EI-MS (70eV): *m*/*z* (%) = 294 (4) [M^+], 238 (5), 225 (2), 149 (4), 135 (10), 121 (14), 108 (34), 93 (46), 79 (100), 67 (66), 55 (32), 41 (48).

Synthesis of (11*Z*,14*Z*,17*Z*)-3,3,4,4-tetradeuteroeicosa-11,14,17-trienoic acid (102)

a) Preparation of [D_4]-3,3,4,4-(11*Z*,14*Z*,17*Z*)-11,14,17-eicosatrienol **101**:

This compound was synthesized according to the general procedure **F**.

Used amounts: 30 mg (0.10 mmol) **100**, 7.4 mg (0.18 mmol) LAH, yield: 85% (29 mg, 0.085 mmol) **101**.

EI-MS (70eV): *m*/*z* (%) = 296 (4) [M^+], 240 (5), 163 (2), 149 (7), 135 (8), 121 (12), 108 (45),

93 (41), 79 (100), 67 (63), 55 (29), 41 (40).

b) Preparation of **102**:

This compound was prepared according to the synthesis of **98**.

Used amounts: 29 mg (0.085 mmol) **101**, 0.200g (0.53 mmol) PDC, yield: 25% (6.6 mg, 0.021 mmol).

R_F = 0.25 (pentane/diethyl ether 3/1).

EI-MS of the TMS-derivative of **102** (70eV): *m/z* (%) = 382 (3) [M^+], 367 (15), 326 (1), 131 (32), 121 (16), 108 (42), 95 (53), 93 (37), 79 (83), 75 (100), 73 (92), 67 (51), 55 (25), 41 (33).

Synthesis of (*Z*)-1,12-heptadecadiene (108)

A suspension of 0.232 g (0.65 mmol) methyltriphenylphosphonium bromide in 10 mL anhydr. DME was prepared, cooled to 0°C, and 0.41 mL (0.65 mmol) of a 1.6M methyl lithium solution in diethyl ether were added dropwise. The mixture was stirred for 30min at rt and a yellow solution was obtained. A solution of 0.155 g (0.65 mmol) (*Z*)-11-hexadecenal in 3 mL anhydr. DME was added and the mixture was stirred overnight. The reaction was quenched with 10 mL H_2O and 10 mL diethyl ether were added. The organic phase was separated and the aqueous phase was washed twice with 10 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane) to provide 0.097g (0.41 mmol) **108** in 63% yield.

R_F = 0.88 (pentane).

RI = 1683.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.90 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.24 – 1.43 (m, 18H, 9*CH_2), 1.97 – 2.07 (m, 6H, 3*CH_2), 4.91 – 5.02 (tddd, J = 1.4 Hz, 2.2 Hz, ^3J = 10.2 Hz, 17.1 Hz, 2H, C<u>HH</u>`), , 5.31 – 5.40 (m, 2H, 2*CH), 5.82 (tdd, ^3J = 6.7 Hz, 10.3, 17.0 Hz, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.4 (CH_2), 27.0 (CH_2), 27.2 (CH_2), 29.0 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (2*CH_2), 29.6 (CH_2), 29.8 (CH_2), 32.0 (CH_2), 33.8 (CH_2), 114.1 (CH_2), 129.9 (2*CH), 139.3 (CH).

EI-MS (70eV): *m/z* (%) = 236 (9) [M^+], 194 (1), 180 (1), 166 (2), 152 (3), 138 (4), 123 (8), 109 (18), 96 (44), 82 (54), 67 (47), 55 (100), 41 (67).

Synthesis of (*Z*)-12-heptadecen-2-ol (110)

This compound was synthesized according to the general procedure **I**.

Used amounts: 0.165 g (0.69 mmol) **109**, 0.68 mL (2.07 mmol) 3M methylmagnesium chloride solution in THF, yield 92% (0.161 g, 0.63 mmol)

R_F = 0.28 (pentane/diethyl ether 3/1).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.90 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.18 (d, ^3J = 6.1 Hz, 3H, CH_3), 1.21 – 1.51 (m, 20H, 10*CH_2), 1.96 – 2.09 (m, 4H, 2*CH_2), 2.17 (s, 1H, OH), 3.74 – 3.84 (m, 1H, CH), 5.30 – 5.40 (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.3 (CH_2), 23.5 (CH_3), 25.8 (CH_2), 26.9 (CH_2), 27.2 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (3*CH_2), 29.8 (CH_2), 32.0 (CH_2), 39.4 (CH_2), 68.2 (CH), 129.2 (CH), 129.9 (CH).

EI-MS (70eV): *m*/*z* (%) = 254 (5) [M^+], 236 (18), 194 (3), 180 (5), 166 (10), 152 (11), 138 (13), 123 (10), 110 (39), 96 (100), 82 (85), 67 (61), 55 (99), 45 (67), 41 (58).

Synthesis of (*Z*)-12-heptadecan-2-one (111)

PCC (0.052 g, 0.24 mmol) were suspended in 5 mL anhydr. CH_2Cl_2 and 0.040 g (0.16 mmol) **110**, dissolved in 2 mL anhydr. CH_2Cl_2, were added. After 2.5h the educt was completely consumed and the crude mixture was directly purified by column chromatography on silica gel (pentane/diethyl ether 5/1) to furnish 0.038 g (15 mmol) **111** in 94% yield.

R_F = 0.45 (pentane/diethyl ether 5/1).

RI = 1898.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.90 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.23 – 1.36 (m, 16H, 8*CH_2), 1.56 (quint, ^3J = 7.2 Hz, 2H, CH_2), 1.96 – 2.04 (m, 4H, 2*CH_2), 2.13 (s, 3H, CH_3), 2.41 (t, ^3J = 7.5 Hz, 2H, CH_2), 5.33 – 5.40 (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.3 (CH_2), 23.9 (CH_2), 26.9 (CH_2), 27.2 (CH_2), 29.2 (2*CH_2), 29.4 (2*CH_2), 29.5 (CH_2), 29.7 (CH_2), 29.8 (CH_3), 32.0 (CH_2), 43.8 (CH_2), 129.8 (2*CH), 209.3 (Cq).

EI-MS (70eV): *m*/*z* (%) = 252 (4) [M^+], 234 (4), 184 (3), 170 (5), 156 (6), 149 (6), 135 (9), 125 (26), 111 (15), 96 (33), 81 (32), 71 (47), 58 (35), 55 (72), 43 (100).

Synthesis of all-*trans*-farnesylacetonitrile (113)

This compound was synthesized according to the general procedure **G**.

Used amounts: 0.324 g (0.42 mL, 8.23 mmol) acetonitrile, 5.1 mL (8.23 mmol) 1.6M *n*-BuLi, 0.802 g (3.30 mmol) **112**, yield: 32% (0.247g, 1.02 mmol).

After the addition of **112** had been completed, the reaction was stirred for 2h at -78°C and subsequent, the mixture was immediately quenched with H_2O.

R_F = 0.39 (pentane/diethyl ether 20/1).

δ_H [ppm](300MHz, $CDCl_3$, TMS): 1.60 (s, 6H, 2*CH_3), 1.65 (d, ^3J = 1.2 Hz, 3H, CH_3),

1.68 (d, ^3J = 1.0 Hz, 3H, CH_3), 1.95 – 2.17 (m, 8H, 4*CH_2), 2.34 – 2.37 (m, 4H, 2*CH_2), 5.06 – 5.18 (m, 3H, 3*CH).

δ_C [ppm](75MHz, $CDCl_3$, TMS): 16.0 (CH_3), 16.2 (CH_3), 17.6 (CH_2), 17.7 (CH_3), 24.0 (CH_2), 25.7 (CH_3), 26.4 (CH_2), 26.7 (CH_2), 39.5 (CH_2), 39.7 (CH_2), 119.6 (Cq), 120.0 (CH), 123.7 (CH), 124.3 (CH), 131.3 (Cq), 135.3 (Cq), 139.1 (Cq).

EI-MS (70eV): *m/z* (%) = 245 (2) [M^+], 230 (1), 202 (4), 176 (3), 148 (2), 136 (15), 123 (8), 108 (4), 93 (10), 81 (40), 69 (100), 53 (12), 41 (43).

Synthesis of all-*trans*-farnesylacetaldehyde (114)

A solution of 0.040 g (0.163 mmol) **113** in 2 mL anhydr. toluene was prepared, cooled to -78°C, and a solution of 0.17 mL (0.210 mmol) DIBALH in toluene was added. The mixture was allowed to warm up to rt and stirring was continued overnight. The mixture was cooled to 0°C and 1 mL 2.5% aqueous H_2SO_4-solution was added. This mixture was stirred for 30min and a white precipitate was formed. This residue was dissolved with 5 mL aqueous disodium tartrate solution and furthermore, 10 mL diethyl ether were added. The organic layer was separated and the aqueous phase was washed twice with 10 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 50/1) to provide 0.038g (0.146 mmol) **114** in 91% yield.

R_F = 0.29 (pentane/diethyl ether 50/1).

RI = 1843.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.60 (m, 6H, 2*CH_3), 1.63 (d, ^3J = 0.9 Hz, 3H, CH_3), 1.68 (d, ^3J = 1.1 Hz, 3H, CH_3), 1.95 – 2.10 (m, 8H, 4*CH_2), 2.30 – 2.36 (m, 2H, CH_2), 2.46 (dt, ^3J = 1.8 Hz, 6.6 Hz, 2H, CH_2) 5.07 – 5.13 (m, 3H, 3*CH), 9.76 (t, ^3J = 1.8 Hz, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 16.0 (2*CH_3), 17.7 (CH_3), 20.8 (CH_2), 25.7 (CH_3), 26.5 (CH_2), 26.7 (CH_2), 39.6 (CH_2), 39.7 (CH_2), 44.0 (CH_2), 122.0 (CH), 124.0 (CH), 124.4 (CH), 131.3 (Cq), 135.1 (Cq), 136.9 (Cq), 202.6 (CH).

EI-MS (70eV): *m/z* (%) = 248 (1) [M^+], 233 (0.4), 205 (2), 177 (2), 161 (4), 136 (25), 121 (9), 107 (6), 93 (26), 69 (100), 55 (26), 41 (51).

Synthesis of *N*-3-methylbutylpyrrole (119)

This compound was synthesized according to the general procedure **J**.

Used amounts: 0.502 g (7.5 mmol) pyrrole, 2.415 g (7.5 mmol) tetrabutylammonium bromide, 0.826 g (8 mmol) 1-bromo-3-methylbutane, yield: 60% (0.603 g, 4.4 mmol).

R_F = 0.87 (pentane/diethyl ether 10/1).

RI = 1066.

δ_H [ppm](200MHz, $CDCl_3$, TMS): 0.93 (d, ^3J = 6.4 Hz, 6H, 2*CH_3), 1.48 – 1.80 (m, 3H, CH, CH_2), 3.89 (t, ^3J = 7.3 Hz, 2H, CH_2), 6.13 (t, *J* = 2.1 Hz, 2H, 2*CH), 6.65 (t, *J* = 2.1 Hz, 2H, 2*CH).

δ_C [ppm](50MHz, $CDCl_3$, TMS): 22.4 (2*CH_3), 25.5 (CH), 40.4. (CH_2), 47.8 (CH_2), 107.8 (2*CH), 120.4 (2*CH).

EI-MS (70eV): *m*/*z* (%) = 137 (40) [M^+], 122 (2), 94 (6), 81 (100), 80 (49), 67 (9), 53 (14), 41 (12).

Synthesis of *N*-pentylpyrrole (120)

This compound was synthesized according to the general procedure **J**.

Used amounts: 1.004 g (15.0 mmol) pyrrole, 4.830 g (15.0 mmol) tetrabutylammonium bromide, 1.652 g (8 mmol) 1-bromo-pentane, yield: 80% (1.644 g, 12.0 mmol).

R_F = 0.90 (pentane/diethyl ether 10/1).

RI = 1105.

δ_H [ppm](300MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 7.0 Hz, 3H, CH_3), 1.20 – 1.44 (m, 4H, 2*CH_2), 1.71 – 1.81 (m, 2H, CH_2), 3.85 (t, ^3J = 7.2 Hz, 2H, CH_2), 6.13 (t, *J* = 2.1 Hz, 2H, 2*CH), 6.64 (t, *J* = 2.1 Hz, 2H, 2*CH).

δ_C [ppm](75MHz, $CDCl_3$, TMS): 13.9 (CH_3), 22.3 (CH_2), 28.9 (CH_2), 31.2 (CH_2), 49.6 (CH_2), 107.7 (2*CH), 120.4 (2*CH).

EI-MS (70eV): *m*/*z* (%) = 137 (70) [M^+], 122 (6), 108 (10), 94 (19), 81 (100), 80 (83), 67 (18), 53 (27), 41 (22).

Synthesis of 2-methylpyrrole (122)

A suspension of 1.14 g (30 mmol) LAH in 25 mL anhydr. THF was prepared, cooled to 0°C, and a solution of 1 g (10 mmol) pyrrole-2-carbaldehyde in 8 mL anhydr. THF was added over a period of 20 min [Liddel *et al.*, 1993]. The mixture was heated to reflux for 36h and then again, the mixture was cooled to 0°C followed by the dropwise addition of 2 mL H_2O and 2 mL aqueous 4M sodium hydroxide solution. The lithium salts were removed by filtration and the filtrate was diluted with 10 mL H_2O and 20 mL CH_2Cl_2. The organic layer was separated and the aqueous phase was washed twice with 20 mL CH_2Cl_2. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by destillation (bp. 130°C at 500 mbar) to provide 0.324 g (4 mmol) **122** in 40% yield.

δ_H [ppm](200MHz, $CDCl_3$, TMS): 2.25 (s, 3H, CH_3), 5.87 – 5.91 (m, 1H, CH), 6.08 – 6.13 (m, 1H, CH), 6.59 – 6.63 (m, 1H, CH), 7.80 (br. s, 1H, NH).

δ_C [ppm](50MHz, $CDCl_3$, TMS): 12.8 (CH_3), 105.8 (CH), 108.3 (CH), 116.2 (CH).

EI-MS (70eV): *m*/*z* (%) = 81 (87) [M^+], 80 (100), 53 (41), 51 (15), 41 (5), 39 (11).

Synthesis of 2-methyl-*N*-3-methylbutylpyrrole (123)

This compound was synthesized according to the general procedure **J**.

Used amounts: 0.267 g (3.3 mmol) **122**, 1.063 g (3.3 mmol) tetrabutylammonium bromide, 0.361 g (3.5 mmol) 1-bromo-3-methylbutane, yield : 17% (0.076 g, 0.5 mmol).

R_F = 0.85 (pentane/diethyl ether 10/1).

RI = 1148.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (d, ^3J = 6.4 Hz, 6H, 2*CH_3), 1.49 – 1.55 (m, 3H, CH, CH_2), 2.10 (s, 3H, CH_3), 3,71 (t, ^3J = 7.5 Hz, 2H, CH_2), 5.77 – 5.79 (m, 1H, CH), 5.96 (t, ^3J = 2.1 Hz, 1H, CH). 6.49 – 6.59 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 11.9 (CH_3), 22.5 (2*CH_3), 25.7 (CH), 40.2 (CH), 44.9 (CH), 106.4 (CH), 106.5 (CH), 119.6 (CH), 128.1 (Cq).

EI-MS (70eV): *m*/*z* (%) = 151 (49) [M^+], 136 (6), 108 (14), 94 (100), 80 (23), 67 (9), 53 (17), 41 (18).

Synthesis of (*Z*)-9-octadecenoic acid amide (139)

This compound was synthesized according to the general procedure **K**.

Used amounts: 0.299 g (1.06 mmol) oleic acid, 0.267 g (2.12 mmol) oxalyl chloride, 50 mL conc. ammonia, yield: 54% (0.161 g, 0.57 mmol).

R_F = 0.16 (pentane/diethyl ether 1/1).

RI = 2391.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 7.0 Hz, 3H, CH_3), 1.21 – 1.42 (m, 22H, 11*CH_2), 1.63 (quint, ^3J = 7.3 Hz, 2H, CH_2), 1.98 – 2.08 (m, 4H, 2*CH_2), 2.30 (t, ^3J = 7.3 Hz, 2H, CH_2), 5.30 - 5.38 (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (CH_3), 22.7 (CH_2), 25.2 (CH_2), 27.2 (2*CH_2), 28.2 (CH_2), 28.7 (CH_2), 29.4 (CH_2), 29.5 (3*CH_2), 29.7 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 35.6 (CH_2), 129.8 (CH), 130.0 (CH), 173.3 (Cq).

EI-MS (70eV): *m*/*z* (%) = 281 (4) [M^+], 264 (2), 238 (3), 198 (1), 184 (2), 154 (4), 140 (4), 126 (10), 112 (8), 98 (9), 83 (9), 72 (66), 59 (100), 55 (34), 41 (30).

Synthesis of (*E*)-9-octadecenoic acid amide (140)

This compound was synthesized according to the general procedure **K**.

Used amounts: 0.299 g (1.06 mmol) oleic acid, 0.267 g (2.12 mmol) oxalyl chloride, 50 mL conc. ammonia, yield: 72% (0.214 g, 0.76 mmol).

R_F = 0.20 (pentane/diethyl ether 1/1).

RI = 2398.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 7.0 Hz, 3H, CH_3), 1.20 – 1.46 (m, 22H, 11*CH_2), 1.68 (quint, ^3J = 7.0 Hz, 2H, CH_2), 1.90 – 1.97 (m, 4H, 2*CH_2), 2.33 (t, ^3J = 7.0 Hz, 2H, CH_2), 5.36 - 5.40 (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (CH_3), 22.7 (CH_2), 27.4 (CH_2), 28.9 (CH_2), 29.1 (2*CH_2), 29.3 (2*CH_2), 29.4 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 31.1 (CH_2), 31.7 (CH_2), 32.0 (CH_2), 32.7 (CH_2), 130.1 (CH), 130.6 (CH), 173.1 (Cq).

EI-MS (70eV): *m*/*z* (%) = 281 (5) [M^+], 264 (2), 238 (3), 198 (1), 184 (2), 154 (3), 140 (4), 126 (9), 112 (8), 98 (9), 83 (11), 72 (66), 59 (100), 55 (34), 41 (29).

Synthesis of *N*,*N*-dimethylaminoethyl α-linoleate (141)

This compound was synthesized according to the general procedure **A**.

Used amounts: 0.207 g (0.74 mmol) α-linoleic acid, 0.065 g (0.74 mmol) *N*,*N*-dimethylaminoethanol, 0.156 g (0.81 mmol) EDC, 0.009 g (0.07 mmol) DMAP, yield: 87% (0.221 g, 0.60 mmol).

RI = 2463.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.9 Hz, 3H, CH_3), 1.24 – 1.41 (m, 14H, 7*CH_2), 1.58 – 1.66 (m, 2H, CH_2), 2.02 - 2.07 (m, 4H, 2*CH_2), 2.28 (s, 6H, 2*CH_3), 2.32 (t, ^3J = 7.6 Hz, 2H, CH_2), 2.56 (t, ^3J = 5.8 Hz, 2H, CH_2), 2.75 – 2.79 (m, 2H, CH_2), 4.17 (t, ^3J = 5.8 Hz, 2H, CH_2), 5.31 – 5.42 (m, 4H, 4*CH).

δ_C [ppm](100 MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.5 (CH_2), 24.9 (CH_2), 25.6 (CH_2), 27.2 (2*CH_2), 29.1 (3*CH_2), 29.3 (CH_2), 29.6 (CH_2), 31.5 (CH_2), 34.2 (CH_2), 45.7 (2*CH_3), 57.8 (CH_2), 62.0 (CH_2), 127.9 (CH), 128.0 (CH), 130.0 (CH), 130.2 (CH), 173.9 (Cq).

EI-MS (70eV): *m*/*z* (%) = 351 (5) [M^+], 113 (1), 99 (2), 71 (28), 58 (100), 43 (8).

Synthesis of α-linoleic acid ethanolamide (142)

This compound was synthesised according to the general procedure **K**.

Used amounts: 0.300 g (1.07 mmol) α-linoleic acid, 0.270 g (2.14 mmol) oxalyl chloride, 0.067 g (1.07 mmol) ethanolamine, yield: 93% (0.323g, 1.00 mmol).

R_F = 0.15 (pentane/diethyl ether 1/1).

RI = 2327.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.89 (t, ^3J = 6.7 Hz, 3H, CH_3), 1.24 – 1.46 (m, 14H, 7*CH_2), 1.56 – 1.64 (m, 2H, CH_2), 2.03 -2.07 (m, 4H, 2*CH_2), 2.27 (t, ^3J = 7.6 Hz, 2H, CH_2), 2.75 – 2.79 (m, 2H, CH_2), 3.41 (t, ^3J = 5.6 Hz, 2H, CH_2), 3.59 (t, ^3J = 5.6 Hz, 2H, CH_2), 5.29 – 5.43 (m, 4H, 4*CH).

δ_C [ppm](100 MHz, $CDCl_3$, TMS): 14.1 (CH_3), 22.6 – 31.6 (11*CH_2), 36.3 (CH_2), 41.2 (CH_2), 60.3 (CH_2), 127.8 (CH), 127.9 (CH), 130.1 (CH), 130.2 (CH), 173.7 (Cq).

EI-MS (70eV): *m/z* (%) = 305 (8) [M^+], 276 (4), 262 (13), 248 (21), 234 (7), 220 (5), 208 (10), 194 (25), 180 (3), 168 (5), 154 (15), 140 (7), 112 (14), 98 (100), 85 (64), 79 (14), 67 (25), 55 (30), 41 (30).

Synthesis of 2-*tert*-butyl-5-methoxycarbonyl-1,3-dioxolan-4-one (144)

A mixture of 5 g (37.5 mmol) malic acid, 5 g (58 mmol) 2,2-dimethylpropanal, 0.625 g (3.65 mmol) *p*-TsOH, and two drops of conc. H_2SO_4 in 50 mL pentane was heated to reflux for 16h under removal of H_2O with a water separator. The suspension was filtered, the residue was taken up in CH_2Cl_2, and washed with 50 mL 8% aqueous H_3PO_4-solution. The organic phase was separated, dried with $MgSO_4$ and ¾ of the solvent were removed under reduced pressure. Then, the remaining solution was kept in a freezer (-20°C) overnight. A white precipitate was formed and the brown solution was removed by decantation to furnish 4.5 g (22.3 mmol) **144** in 60% yield.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.97 (s, 9H, 3*CH_3), 2.96 (t, ^3J = 4.6 Hz, 2H, CH_2), 4.63 (dt, ^3J = 4.7 Hz, ^4J = 1.8 Hz, 1H, CH), 5.35 (d, ^4J = 1.8 Hz, 1H, CH), 10.7 (br. s, 1H, OH).

δ_C [ppm](100 MHz, $CDCl_3$, TMS): 23.2 (3*CH_3), 35.8 (CH_2), 35.9 (Cq), 70.7 (CH), 111.3 (CH), 172.4 (Cq), 175.4 (Cq).

Synthesis of 2-*tert*-butyl-5-(2-methoxycarbonyl-ethyl)-5-(2-methoxycarbonyl-1-methylethyl)-1,3-dioxolan-4-one (145)

A solution of 0.540 g (2.67 mmol) **144** in 30 mL anhydr. THF was prepared, cooled to -78°C, and 5.67 mL (5.67 mmol) of a 1M LiHMDS-solution in THF was added. The mixture was stirred for 1h, followed by the addition of 0.53 mL (4.80 mmol) methyl 2-bromopropionate. The mixture was allowed to warm up to -20°C and stirring was continued for 1h. Then, the mixture was warmed up to -10°C, 50 mL H_2O were added, and the mixture was diluted with 50 mL CH_2Cl_2. The aqueous phase was acidified (pH = 2) with 2M aqueous HCl-solution and the organic phase was separated. The aqueous layer was washed twice with 50 mL CH_2Cl_2. The organic phases were combined, dried

with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was filtered over silica gel (acetone) and a fraction (0.585 g) containing the product, a brominated sideproduct (dimethyl 2-bromo-3-methyl-butandionate) and excess methyl 2-bromopropionate was obtained. Excess methyl 2-bromopropionate was removed by destillation (1mbar/140°C).

This crude fraction was dissolved in 5 mL anhydr. methanol, cooled to 0°C, and 0.703 g (5 mmol) BF_3-diethyl ether complex were added. The mixture was allowed to warm up to rt and stirring was continued for 24h. The mixture was quenched with 10 mL H_2O and diluted with 10 mL CH_2Cl_2. The organic phase was separated and the aqueous phase was washed twice with 5 mL CH_2Cl_2. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (CH_2Cl_2) to provide 0.044 g of a fraction consisting of **145** and dimethyl 2-bromo-3-methyl-butandionate in a 1:1-ratio.

EI-MS (70eV): *m*/*z* (%) = 271 (1) [M^+-31], 245 [M^+-57] (29), 199 (27), 185 (8), 167 (8), 157 (100), 125 (6), 115 (29), 101 (20), 87 (12), 70 (10), 57 (25), 41 (17).

Synthesis of trimethyl methylcitrate (146)

Crude **145** was dissolved in 2 mL anhydr. methanol and 0.050 g (0.71 mmol) $NaHCO_3$ were added. The mixture was stirred overnight at rt, 3 mL H_2O were added, and the mixture was diluted with 3 mL CH_2Cl_2. The organic phase was separated and the aqueous phase was washed twice with 3 mL CH_2Cl_2. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure to yield 0.025 g crude product. The mixture comprised both diastereoisomers in a 1:1-ratio.

RI = 1518 (1.diastereomer); *RI* = 1527 (2.diastereomer).

EI-MS (70eV): *m*/*z* (%) = 189 (12) [M^+-59], 157 (100), 125 (8), 115 (50), 101 (23), 88 (22), 83 (6), 69 (6), 59 (27), 43 (11).

Synthesis of (*S*)-*β*-octanoylbutyrolactone (147)

This compound was synthesized according to the general procedure **A**.

Used amounts: 0.050 g (0.49 mmol) (*S*)-*β*-hydroxybutyrolactone, 0.071 g (0.49 mmol) octanoic acid, 0.103 g (0.54 mmol) EDC, 0.006 g (0.005 mmol) DMAP, yield: 72% (0.080 g, 0.35 mmol) after filtration on silica gel with diethyl ether.

RI = 1809.

$[\alpha]_D^{22}$ = -5.24 ± 0.02 (*c* = 1.0, CH_2Cl_2).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 7.0 Hz, 3H, CH_3), 1.23 – 1.37 (m, 8H, 4*CH_2),

1.58 – 1.67 (m, 2H, CH_2), 2.34 (t, ^3J = 7.4 Hz, 2H, CH_2), 2.60 (dd, ^3J = 1.5 Hz, ^2J = 18.5 Hz, 1H, CHH`), 2.87 (dd, $^3J$ = 6.8 Hz, $^2J$ = 18.5 Hz, 1H, CHH`), 4.35 (dd, ^3J = 1.5 Hz, ^2J = 11.1 Hz, 1H, CHH`), 4.52 (dd, $^3J$ = 4.9Hz, $^2J$ = 11.0 Hz, 1H, CHH`), 5.44 (dddd, ^3J = 1,5 Hz, 1.5 Hz, 4.9 Hz, 6.6 Hz, 1H, CH).

δ_C [ppm](100 MHz, $CDCl_3$, TMS): 14.0 (CH_3), 22.5 (CH_2), 24.7 (CH_2), 28.8 (CH_2), 28.9 (CH_2), 31.5 (CH_2), 34.0 (CH_2), 34.5 (CH_2), 69.5 (CH), 73.1 (CH_2), 173.2 (Cq), 174.6 (Cq).

EI-MS (70eV): *m*/*z* (%) = 228 (0.2) [M^+], 199 (3), 185 (8), 171 (3), 157 (17), 144 (19), 127 (25), 98 (6), 85 (100), 57 (43), 41 (25).

Synthesis of ethyl 3-oxo-docosanoate (154)

This compound was synthesized according to the general procedure **L**.

Used amounts: 1.5 g (5.07 mmol) **153**, 0.638 g (0.58 mL, 5.58 mmol) ethyl diazoacetate, 0.096 g (0.51 mmol) $SnCl_2$, yield: 78% (1.519 g, 3.95 mmol).

R_F = 0.27 (pentane/diethyl ether 10/1).

The keto isomer is predominant in solution.

Equilibrium ratio: keto/enol 10:1.

The ^{13}C nmr spectra describes only the data of the keto isomer.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 6.9 Hz, $3H^K$, $3H^E$, 2*CH_3), 1.26 – 1.33 (m, $35H^K$, $35H^E$, 32* CH_2, 2* CH_3), 1.55 – 1.61 (m, $2H^K$, $2H^E$, 2* CH_2), 2.18 (t, ^3J = 7.7 Hz, $2H^E$, CH_2), 2.53 (t, ^3J = 7.4 Hz , $2H^K$, CH_2), 3.42 (s, $2H^K$, CH_2), 4.20 (q, ^3J = 7.1 Hz, $2H^K$, CH_2), 4.26 (q, ^3J = 7.1 Hz, $2H^K$, CH_2), 4.97 (s, $1H^E$, CH), 12.10 (s, 1H, OH^E).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (2*CH_3), 22.7 – 29.7 (16*CH_2), 31.9 (CH_2), 43.0 (CH_2), 49.3 (CH_2), 61.3 (CH_2), 167.2 (C_q), 202.9 (C_q).

Synthesis of 1,3-docosanediol (155)

A mixture of 0.200 g (0.52 mmol) **154** and 0.050 g (1.31 mmol) $NaBH_4$ in 5 mL dioxane was prepared and heated to reflux [Soai and Oyamada, 1984]. Anhydr. methanol (0.790 g, 1 mL, 24.7 mmol) was added and heating was continued for 2h. After the mixture was cooled down to rt, 10 mL H_2O and 5 mL 2M aequous HCl were added. Furthermore, the mixture was diluted with 10 mL diethyl ether. The organic phase was separated and the aqueous phase was washed twice with 10 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 1/1) to provide 0.125 g (0.37 mmol) **155** in 71%

yield.

R_F = 0.07 (pentane/diethyl ether 1/1).

EI-MS of the TMS-derivative of **155** (70eV): *m/z* (%) = 471 (5) [M^+- CH_3], 443 (8), 381 (4), 369 (68), 219 (100), 147 (51), 129 (20), 103 (61), 73 (52), 57 (19), 43 (25).

EI-MS of the on-column-derivative of **155** (70eV): *m/z* (%) = 383 (4) [M^+- CH_3], 131 (100), 101 (4), 75 (5), 57 (3), 43 (4).

NMR-spectra were not recorded because the solid white compound did not dissolve in numerous organic solvents at rt.

Synthesis of methyl (*S*)-3-hydroxy-docosanoate (156)

Compound **154** (0.245 g, 0.64 mmol) was dissolved in 10 mL anhydr. methanol. Then, 1 mL 4mM solution of (*S*)-[NH_2Et_2][{RuCl-(*R*)-(BINAP)}$_2$(μ-Cl)$_3$] in THF and 3 drops conc. HCl were added. The resulting mixture was hydrogenated for 48h at 80°C and 15 bar. The solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/diethyl ether 10/1) to provide **156** in 77% yield (0.180 g, 0.49 mmol). The ethyl ester was transesterified to a methyl ester during this reaction.

R_F = 0.11 (pentane/diethyl ether 10/1).

e.r. = *S*/*R* 93:7

$[\alpha]_D^{21}$ = 1.5 ± 0.1 (*c* = 1.2, CH_2Cl_2).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.88 (t, ^3J = 6.9 Hz, 3H, CH_3), 1.21 – 1.56 (m, 36H), 2.41 (dd, ^3J = 9.0 Hz, ^2J = 16.4 Hz, 1H, C<u>H</u>H´), 2.52 (dd, ^3J = 3.1 Hz, ^2J = 16.4 Hz, 1H, CH<u>H</u>´), 2.89 (br s, 1H, OH), 3.71 (s, 3H, CH_3), 3.99 – 4.03 (m, 1H, CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.1 (CH_3), 22.7 (CH_2), 25.5 (CH_2), 29.4 – 29.7 (14*CH_2), 31.9 (CH_2), 36.5 (CH_2), 41.1 (CH_2), 51.7 (CH_3), 68.0 (CH), 173.5 (C_q).

EI-MS (70eV): *m/z* (%) = 352 (2) [M^+], 320 (7), 103 (100), 95 (6), 83 (10), 71 (27), 57 (24), 55 (2), 43 (52).

Synthesis of (*S*)-1,3-docosanediol (157)

A solution of **156** (0.145 g, 0.38 mmol) in 5 mL anhydr. diethyl ether was prepared and was added dropwise to a suspension of 0.026 g (0.67 mmol) LAH in 5 mL anhydr. diethyl ether. This mixture was heated to reflux for 2h and stirring was continued at rt overnight. The mixture was diluted with 5 mL H_2O and quenched with 5 mL 2M aqueous HCl. Furthermore, 10 mL diethyl ether were added and the organic phase was separated. The aqueous phase was washed twice with 10 mL diethyl ether. The organic phases were combined, dried with $MgSO_4$, and the solvents were

removed under reduced pressure. The crude product was purified by column chromatography on silica gel (pentane/diethyl ether 1/1) to provide 0.130 g (0.37 mmol) **157** in 97% yield.

R_F = 0.07 (pentane/diethyl ether 1/1).

$[\alpha]_D^{21}$ = 0.8 ± 0.1 (*c* = 1.0, CH_2Cl_2).

The mass spectrometric data correspond to those of **155**.

Synthesis of (3*S*,5*S*)-3,5-heptanediol (162)

A solution of 0.945 g (7.38 mmol) 3,5-heptandione in 5 mL anhydr. methanol was prepared. Then, 1 mL 4mM solution of (*S*)-$[NH_2Et_2][\{RuCl\text{-}(R)\text{-}(BINAP)\}_2(\mu\text{-}Cl)_3]$ in THF and 2 drops conc. HCl were added. The resulting mixture was hydrogenated for 20h at 60°C and 40 bar. The solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/diethyl ether 1/1) to provide **190** in 72% yield (0.705 g, 5.34 mmol).

R_F = 0.10 (pentane/diethyl ether 1/1).

$[\alpha]_D^{23}$ = 13.0 ± 0.1 (*c* = 3.8, $CHCl_3$).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.95 (t, ^3J = 7.5 Hz, 6H, 2*CH_3), 1.55 (m, 6H, 3*CH_2), 3.20 (br. s, 2H, OH), 3.85 ppm (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 10.1 (2*CH_3), 30.2 (2*CH_2), 41.4 (CH_2), 70.7 (2*CH).

EI-MS of the TMS-derivative of **162** (70eV): *m*/*z* (%) = 261 (1) [M^+-CH_3], 247 (2), 205 (5), 186 (3), 171 (1), 157 (13), 145 (1), 131 (100), 115 (3), 101 (5), 85 (1), 59 (5), 45 (7).

Synthesis of ethyl 2-butyryl-3-oxo-hexanoate

This compound was synthesized according to the general procedure **M**.

Used amounts: 7.14 g (75 mmol) $MgCl_2$, 11.85 g (12.1 mL, 75 mmol) ethyl 3-oxo hexanoate, 11.85 g (12.2 mL, 150 mmol) pyridine, 7.95 g (7.8 mL, 75 mmol) butyric acid chloride, yield: 86% (14.77 g, 64.8 mmol).

R_F = 0.45 (pentane/diethyl ether 20/1).

The nmr describes only the data of the enolic form.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.96 (t, ^3J = 7.4 Hz, 6H, 2*CH_3), 1.35 (t, ^3J = 7.1 Hz, 3H, CH_3), 1.68 (sex, ^3J = 7.5 Hz, 4H, 2*CH_2), 2.59 (t, ^3J = 7.6 Hz, 4H, 2*CH_2), 4.28 (q, ^3J = 7.1 Hz, 2H, CH_2).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 13.8 (2*CH_3), 14.1 (CH_3), 19.2 (2*CH_2), 39.4 (2*CH_2), 60.8 (CH_2), 108.8 (Cq), 167.5 (Cq), 197.8 (2*Cq).

EI-MS (70eV): *m*/*z* (%) = 228 (1), [M^+], 185 (77), 157 (11), 139 (16), 126 (6), 113 (13), 97 (7), 84 (6), 71 (100), 55 (9), 43 (57).

Synthesis of 4,6-nonandione

This compound was synthesized according to the general procedure **N**.

Used amounts: 14.77 g (64.8 mmol) ethyl 2-butyryl-3-oxo-hexanoate, 3.77 g (64.8 mmol) NaCl, 3.9 mL H_2O, 65 mL DMSO, 5h reflux, yield: 76% (7.75 g, 49.7 mmol).

R_F = 0.08 (pentane/diethyl ether 40/1).

The enol isomer predominates in solution.

Equilibrium ratio: enol/keto 4.6:1.

δ_H [ppm](200MHz, $CDCl_3$, TMS): 0.95 (t, ^3J = 7.3 Hz, 6H, 2*CH_3^E), 0.97 (t, ^3J = 7.3 Hz, 6H, 2*CH_3^K), 1.66 (sex, ^3J = 7.1 Hz, 8H, 2*CH_2^E , 2*CH_2^K), 2.26 (t, ^3J = 7.5 Hz, 4H, 2*CH_2^E), 2.49 (t, ^3J = 7.3 Hz, 4H, 2*CH_2^K), 5.49 (s, 1H, CH^E), 15.47 (s, 1H, OH^E).

δ_C [ppm](50MHz, $CDCl_3$, TMS): 13.7 (2*CH_3), 19.1 (2*CH_2), 40.3 (2*CH_2), 99.2 (Cq), 194.3 (2*Cq).

EI-MS (70eV): *m*/*z* (%) = 156 (18) [M^+], 141 (4), 128 (6), 113 (100), 71 (33), 69 (9), 43 (32), 41 (19), 39 (11).

Synthesis of (4*S*,6*S*)-4,6-nonanediol (163)

A solution of 0.945 g (6.06 mmol) 4,6-nonandione in 13 mL anhydr. methanol was prepared. Then, 2 mL 4mM solution of (*S*)-[NH_2Et_2][{RuCl-(*R*)-(BINAP)}$_2$(μ-Cl)$_3$] in THF and 4 drops conc. HCl were added. The resulting mixture was hydrogenated for 20h at 80°C and 10 bar. The solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/diethyl ether 2/1) to provide **163** in 10% yield (0.093 g, 0.58 mmol).

R_F = 0.05 (pentane/diethyl ether 2/1).

$[\alpha]_D^{23}$ = 9.0 ± 0.5 (*c* = 2.0, $CHCl_3$).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.93 (t, ^3J = 7.0 Hz, 6H, 2*CH_3), 1.31 – 1.56 (m, 8H, 4*CH_2), 1.60 – 1.58 (m, 2H, CH_2), 3.91 – 3.97 (m, 2H, 2*CH):

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.4 (2*CH_3), 19.3 (2*CH_2), 39.9 (2*CH_2), 42.6 (CH_2), 69.4 (2*CH).

EI-MS (70eV) of TMS-derivative of **163**: *m*/*z* (%) = 261 (1) [M^+- 43], 219 (6), 171 (25), 145 (100), 131 (7), 101 (13), 73 (91), 59 (11), 45 (14).

Synthesis of ethyl 4-methyl-3-oxo-pentanoate

This compound was synthesized according to the general procedure **L**.

Used amounts: 5.92 g (80 mmol) 2-methylpropanal, 1.52 g (8 mmol) $SnCl_2$,

10.04 g (88 mmol) ethyl diazoacetate, yield: 72% (9.13 g, 58 mmol).

R_F = 0.25 (pentane/diethyl ether 7/1).

The keto isomer predominates in solution.

Equilibrium ratio: keto/enol 8.5:1.

The ^{13}C nmr spectra describes only the data of the keto isomer.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.94 (d, ^3J = 6.9 Hz, 6H, 2*CH_3^E), 1.16 (d, ^3J = 6.9 Hz, 6H, 2*CH_3^K), 1.30 (t, ^3J = 7.1 Hz, 6H, CH_3^E, CH_3^K), 2.43 (sept, ^3J = 6.9 Hz, 1H, CH^E), 2.74 (sept, ^3J = 6.9 Hz, 1H, CH^K), 3.51 (s, 2H, CH_2^K), 4.21 (q, ^3J = 7.1 Hz, 4H, CH_2^E, CH_2^K), 5.00 (s, 1H, CH^E), 12.16 (s, 1H, OH^E).

δ_C [ppm](100MHz, $CDCL_3$, TMS): 13.7 (CH_3), 17.5 (2*CH_3), 40.8 (CH), 46.8 (CH_2), 60.9 (CH_2), 167.0 (C_q), 206.2 (C_q).

EI-MS (70eV): *m/z* (%) = 158 (14) [M^+], 115 (19), 112 (11), 97 (2), 87 (5), 71 (43), 69 (22), 43 (100), 41 (38), 39 (18).

Synthesis of ethyl 2-(2-methylpropyryl)-4-methyl-3-oxo-pentanoate

This compound was synthesized according to the general procedure **M**.

Used amounts: 4.76 g (50 mmol) $MgCl_2$, 7.90 g (50 mmol) ethyl 4-methyl-3-oxo pentanoate, 7.82 g (8.1 mL, 100 mmol) pyridine, 5.30 g (5.3 mL, 50 mmol) 2-methylpropionic acid chloride, yield: 93% (10.56 g, 46.3 mmol).

Product was purified by filtration with diethyl ether on silica gel.

Only the enolic form was visible in the nmr spectra.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.16 (d, ^3J = 6.8 Hz, 12H, 4*CH_3), 1.34 (t, ^3J = 7.2 Hz, 3H, CH_3), 3.00 (sept, ^3J = 6.8 Hz, 2H, 2*CH), 4.28 (q, ^3J = 7.2 Hz, 2H, CH_2), 12.14 (s, 1H, OH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.0 (CH_3), 19.4 (4*CH_3), 34.6 (2*CH), 61.0 (CH_2), 107.3 (C_q), 167.9 (C_q), 200.9 (2*C_q).

Synthesis of 2,6-dimethyl-3,5-heptandione

This compound was synthesized according to the general procedure **N**.

Used amounts: 10.56 g (46.3 mmol) ethyl 2-(2-methylpropyryl)-4-methyl-3-oxopentanoate, 2.71 g (46.3 mmol) NaCl, 2.8 mL H_2O, 46 mL DMSO, 7h reflux, yield: 64% (4.67 g, 29.6 mmol).

R_F = 0.74 (pentane/diethyl ether 4/1).

Only the enolic form was visible in the nmr spectra.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.15 (d, ^3J = 7.0 Hz, 12H, 4*CH_3),

2.48 (sept, ^3J = 6.9 Hz, 2H, 2*CH), 5.52 (s, 1H, CH), 15.65 (s, 1H, OH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 19.6 (4*CH_3), 37.0 (2*CH), 95.2 (CH), 199.3 (2*C_q).

EI-MS (70eV): *m*/*z* (%) = 156 (27) [M^+], 113 (100), 95 (6), 85 (5), 71 (15), 57 (18), 43 (66).

Synthesis of (3*S*,5*S*)-2,6-dimethyl-3,5-heptanediol (164)

A solution of 0.587 g (3.76 mmol) 2,6-dimethyl-3,5-heptanedione in 8 mL anhydr. methanol was prepared and 5 mg [*S*(-)-BINAP]-chloro-(*p*-cymene)-ruthenium chloride were added. The resulting mixture was hydrogenated for 70h at 80°C and 40 bar. The solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/diethyl ether 2/1) to provide **192** in 52% yield (0.313 g, 1.96 mmol).

R_F = 0.10 (pentane/diethyl ether 2/1).

$[\alpha]_D^{23}$ = 54.2 ± 0.1 (*c* = 2.6, MeOH).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 0.91 (d, ^3J = 6.8 Hz, 6H, 2*CH_3), 0.96 (d, ^3J = 6.7 Hz, 6H, 2*CH_3), 1.60 (dd, J_1 = 5.2 Hz, J_2 = 6.5 Hz, 2H, CH_2), 1.71 (oct, ^3J = 6.7 Hz, 2H, 2*CH), 2.35 (s, 2H, 2*OH), 3.65 (q, ^3J = 6.0 Hz, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 18.0 (2*CH_3), 18.7 (2*CH_3), 33.7 (2*CH), 36.5 (CH_2), 74.2 (2*CH).

EI-MS (70eV) of the TMS-derivative of **164**: *m*/*z* (%) = 261 (1) [M^+- 43], 219 (2), 171 (4), 145 (100), 129 (10), 101 (11), 73 (87), 59 (8), 43 (12).

Synthesis of ethyl 2-cyclopentyryl-3-cyclopentyl-3-oxo-propionate

a) Preparation of ethyl 3-cyclopentyl-3-oxo-propionate:

This compound was synthesized according to the general procedure **L**.

Used amounts: 1 g (10.2 mmol) cyclopentylcarbaldehyde, 0.19 g (1 mmol) $SnCl_2$, 1.28 g (11 mmol) ethyl diazoacetate, yield: 73% (1.36 g, 7.4 mmol).

Product was purified by filtration on silica gel (pentane/diethyl ether 15/1).

EI-MS (70eV): *m*/*z* (%) = 184 (11) [M^+], 143 (18), 115 (9), 97 (50), 81 (8), 69 (100), 55 (12), 41 (46).

b) Preparation of ethyl 2-cyclopentyryl-3-cyclopentyl-3-oxo-propionate:

This compound was synthesized according to the general procedure **M**.

Used amounts: 1.69 g (7.4 mmol) $MgCl_2$, 1.30 g (7.4 mmol) ethyl 3-cyclopentyl-3-oxo-propionate, 1.16 g (1.2 mL, 14.8 mmol) pyridine, 0.98 g (0.90 mL, 7.4 mmol) 2-cyclopentylacetic acid chloride, yield: 63% (1.32 g , 4.7 mmol).

R_F = 0.23 (pentane/diethyl ether 30/1).

The enol isomer predominates in solution.

Equilibrium ratio: enol/keto 2.8:1.

The ^{13}C nmr spectra describes only the data of the enol isomer.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.29 – 1.36 (m, 6H, CH_3^E, CH_3^K), 1.54 – 1.94 (m, 32H, 8* CH_2^E, 8* CH_2^K), 2.73 – 2.89 (m, 2H, 2*CH^K), 3.12 (quint, ^3J = 8.0 Hz, 2H, 2*CH^E), 4.06 (s, 1H, CH^K), 4.21 – 4.30 (m, 4H, CH_2^E, CH_2^K), 17.63 (s, 1H, OH^E).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 14.4 (CH_3), 26.6 (4*CH_2), 31.2 (4*CH_2), 45.8 (2*CH), 61.2 (CH_2), 109.1 (C_q), 168.5 (C_q), 199.8 (2*C_q).

Synthesis of 1,3-dicyclopentyl-1,3-propandione

This compound was synthesized according to the general procedure **N**.

Used amounts: 1.32 g (4.7 mmol) ethyl 2-cyclopentyryl-3-cyclopentyl-3-oxo-propionate, 0.29 g (5 mmol) NaCl, 0.3 mL H_2O, 5 mL DMSO, yield: 53% (0.511 g, 2.5 mmol).

R_F = 0.25 (pentane/diethyl ether 40/1).

The enol isomer is predominant in solution.

Equilibrium ratio: enol/keto 5.1:1.

The ^{13}C nmr spectra describes only the data of the enol isomer.

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.56 – 1.90 (m, 32H, 8*CH_2^E, 8*CH_2^K), 2.66 (quint, ^3J = 7.9 Hz, 2H, 2*CH^E), 2.98 (quint, ^3J = 8.0 Hz, 2H, 2*CH^K), 3.66 (s, 2H, CH_2^K), 5.53 (s, 1H, CH^E), 15.65 (s, 1H, OH^E).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 25.9 (4*CH_2), 30.3 (4*CH_2), 47.5 (2*CH), 97.1 (CH), 197.0 (2*C_q).

EI-MS (70eV): *m*/*z* (%) = 208 (25) [M^+], 167 (15), 139 (100), 121 (7), 97 (29), 83 (2), 69 (56), 55 (19), 41 (34).

Synthesis of (1*S*,3*S*)-1,3-dicylopentyl-1,3-propanediol (165)

A solution of 0.350 g (1.68 mmol) 1,3-dicyclopentyl-1,3-propandione in 10 mL anhydr. methanol was prepared. Then, 2 mL 4mM solution of (*S*)-$[NH_2Et_2][\{RuCl\text{-}(R)\text{-}(BINAP)\}_2(\mu\text{-}Cl)_3]$ in THF and 4 drops conc. HCl were added. The resulting mixture was hydrogenated for 20h at 80°C and 10 bar. The solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/diethyl ether 2/1) to provide 0.l70 g (0.80 mmol) **165** in 48% yield.

R_F = 0.08 (pentane/diethyl ether 3/1).

$[\alpha]_D^{23}$ = - 7.2 ± 0.2 (*c* = 2.6, $CHCl_3$).

δ_H [ppm](400MHz, $CDCl_3$, TMS): 1.09 – 1.71 (m, 16H, 8*CH_2), 1.81 – 2.00 (m, 4H, 2*CH, 2*CH_2), 2.42 (s, 2H, 2*OH), 3.71 (m, 2H, 2*CH).

δ_C [ppm](100MHz, $CDCl_3$, TMS): 25.6 (2*CH_2), 25.7 (2*CH_2), 29.2 (2*CH_2), 29.3 (2*CH_2), 40.1 (CH_2), 46.2 (2*CH), 74.1 (2*CH).

EI-MS (70eV) of the TMS-derivative of **165**: *m*/*z* (%) = 287 (1) [M^+ - C_5H_9], 245 (2), 217 (1), 197 (5), 171 (100), 147 (8), 117 (4), 103 (9), 81 (13), 73 (41).

Synthesis of (1*R*,3*R*)-1,3-diphenyl-1,3-propanediol (166)

A solution of 1.251 g (5.58 mmol) 1,3-diphenyl-1,3-propandione in 15 mL anhydr. methanol was prepared. Then, 2 mL 4mM solution of (*S*)-[NH_2Et_2][{RuCl-(*R*)-(BINAP)}$_2$(μ-Cl)$_3$] in THF and 4 drops conc. HCl were added. The resulting mixture was hydrogenated for 24h at 80°C and 20 bar. The solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/diethyl ether 3/1) to provide **194** in 10% yield (0.133 g, 0.58 mmol).

R_F = 0.08 (pentane/diethyl ether 3/1).

$[\alpha]_D^{22}$ = 46.1 ± 0.1 (*c* = 2.2, MeOH).

δ_H [ppm](400MHz, [D_4]-MeOH, TMS): 2.05 (t, ^3J = 6.5 Hz, 2H, CH_2), 4.90 (t, ^3J = 6.5 Hz, 2 H, 2*CH) 7.35 (m, 10 H, 10*CH).

δ_C [ppm](100MHz, [D_4]-MeOH, TMS): 49.7 (CH_2), 71.8 (2*CH), 126.9 (4*CH), 128.2 (2*CH), 129.4 (4*CH), 146.6 (2*Cq).

EI-MS of the TMS-derivative of **194** (70eV): *m*/*z* (%) = 295 (1) [M^+-C_6H_5], 282 (25), 253 (8), 193 (4), 179 (100), 165 (4), 147 (17), 133 (3), 119 (10), 104 (9), 89 (1), 77 (3), 73 (55), 59 (2), 45 (6).

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10. Abbreviations

AA	Amino acid
AC	Absolute configuration
AcCl	Acetyl chloride
ATP	Adenosin triphosphate
BINAP	2,2`-Bis(diphenylphosphino)-1,1`-binaphthalin
br. s	broad singulett
cAMP	Cyclic adenosyl monophosphate
CD	Circular dichroism
CDA	Chiral derivatising agent
CNS	Cerebral nervous system
CSP	Chiral stationary phase
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DG	Dufour gland
DIBALH	Diisobutylaluminium hydride
DMAP	Dimethylamino pyridine
DMAPP	Dimethylallylpyrophosphate
DMDS	Dimethyl disulfide
DME	Dimethoxyethane
DMF	Dimethylformamid
DNPU	3,5-Dinitrophenylcarbamate
d.r.	Diastereomeric ratio
EDC	*N*-(3-Dimethylaminopropyl)-*N*`-ethyl-carbodiimide
ee	Enantiomeric excess
equv.	Equivalent
e.r.	Enantiomeric ratio
FAA	Fatty acid amide
$FADH_2$	Flavin adenine dinucleotide
HS	Headspace
IPP	*Iso*-pentenylpyrophosphate
J	coupling constant
JH	Juvenile hormone
LAH	Lithium aluminium hydride

m	Multiplett
MIM	Multiple ion monitoring scan mode
MOP	Monophosphine ligand
MS	Mass spectrometry
MsCl	Methanesulfonyl chloride
MSTFA	*N*-methyl-*N*-trimethylsilyl-trifluoroacetamide
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate) reduced
NAE	*N*-acyl ethanolamine
NaHMDS	Sodium hexadimethylsilylazide
OBP	Odorant-binding protein
OEA	Oleoylethanolamide
OLSA	Open loop stripping analysis
ORP	Optical rotation power
PBAN	Pheromone biosynthesis activating neuropeptide
PCC	Pyrdinium chlorochromate
PDC	Pyridinium dichromate
PLP	Pyridoxal phosphate
PUFA	Polyunsaturated acids
p-TsOH	*para*-Toluensulfonic acid
q	Quartett
quint	Quintett
RC	Relative configuration
RI	Retention index
R_S	Chromatographic resolution, defined as $[(t_{R1} - t_{R2}) / 0.5*(\omega_1 + \omega_2)]$
rt	Room temperature
SAM	S-adenosyl methionine
sex	sextett
SIM	Single ion mode
t	Triplett
THF	Tetrahydrofurane
TIC	Total ion chromatogram
$Ti(OiPr)_4$	Titanium tetraisopropylate
TLC	Thin layer chromatography
TMS	Trimethylsilyl

TMSH	Trimethylsulfonium hydroxide
TMSI	Trimethylsilyl iodide
TPP	Thiamine diphosphate
α	Chromatographic separation factor, defined as ratio of t_{R1} / t_{R2}
$[a]_D^T$	Optical rotation power
δ	Chemical shift in NMR
ω	Peak width

Stephan Goller

Persönliche Daten

Geburtsdatum:	17.12.1977
Geburtsort:	Hildesheim, Niedersachsen
Nationalität:	deutsch
Beruf:	Chemiker

Ausbildungsdaten

06/1997	Allgemeine Hochschulreife, erworben am Gymnasium Josephinum in Hildesheim
09/1997 – 09/1998	Zivildienst in einer Werkstatt für Menschen mit geistiger und körperlicher Behinderung
10/1998 – 09/2000	Grundstudium Chemie an der Technischen Universität Braunschweig
10/2000 – 10/2002	Hauptstudium Chemie an der Technischen Universität Braunschweig
12/2002 – 08/2003	Diplomarbeit in der Arbeitsgruppe von Prof. Dr. Schulz am Institut für Organische Chemie der Technischen Universität Braunschweig, Titel: Chirale Derivatisierungsreagentien für die gaschromatographische Analyse aliphatischer Diole
09/2003 – 01/2008	Promotion in der Arbeitsgruppe von Prof. Dr. Schulz, Titel: Chemical Signals in Arthropods – New structural Motifs

Beruflicher Werdegang

10/2000 – 07/2003	Studentische Hilfskraft im Praktikum Organische Chemie für Chemiker
09/2003 – 03/2007	Wissenschaftlicher Assistent am Institut für Organische Chemie an der Technischen Universität Braunschweig
04/2007 – 07/2007	Tutor am Institut für Organische Chemie an der Technischen Universität Braunschweig
seit 09/2007	Wissenschaftlicher Angestellter bei Dr. U. Noack im Institut für Angewandte Biologie in Sarstedt, Niedersachsen